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NEWS	12	Apr 17 Polymer searching in REGISTRY enhanced
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NEWS	14	Apr 21 New current-awareness alert (SDI) frequency in WPIDS/WPINDEX/WPIX
NEWS	15	Apr 28 RDISCLOSURE now available on STN
NEWS	16	May 05 Pharmacokinetic information and systematic chemical names added to PHAR
NEWS	17	May 15 MEDLINE file segment of TOXCENTER reloaded
NEWS	18	May 15 Supporter information for ENCOMPAT and ENCOMPLIT updated
NEWS	19	May 19 Simultaneous left and right truncation added to WSCA
NEWS	20	May 19 RAPRA enhanced with new search field, simultaneous left and right truncation
NEWS	21	Jun 06 Simultaneous left and right truncation added to CBNB
NEWS	22	Jun 06 PASCAL enhanced with additional data
NEWS	23	Jun 20 2003 edition of the FSTA Thesaurus is now available
NEWS	24	Jun 25 HSDB has been reloaded
NEWS	25	Jul 16 Data from 1960-1976 added to RDISCLOSURE
NEWS	26	Jul 21 Identification of STN records implemented
NEWS	27	Jul 21 Polymer class term count added to REGISTRY
NEWS	28	Jul 22 INPADOC: Basic index (/BI) enhanced; Simultaneous Left and Right Truncation available
NEWS EXPRESS		April 4 CURRENT WINDOWS VERSION IS V6.01a, CURRENT MACINTOSH VERSION IS V6.0b(ENG) AND V6.0Jb(JP), AND CURRENT DISCOVER FILE IS DATED 01 APRIL 2003
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SINCE FILE	TOTAL
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=> s modified food allergen

L1 0 MODIFIED FOOD ALLERGEN

=> s allergen

L2 109688 ALLERGEN

=> s l2 and food

L3 11751 L2 AND FOOD

=> s l3 modified

MISSING OPERATOR L3 MODIFIED

The search profile that was entered contains terms or nested terms that are not separated by a logical operator.

=> s l3 and modified

L4 431 L3 AND MODIFIED

=> s l4 and nucleotide

L5 0 L4 AND NUCLETOIDE

=> s l4 and nucleotide

L6 5 L4 AND NUCLEOTIDE

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PROCESSING COMPLETED FOR L6

L7 4 DUP REMOVE L6 (1 DUPLICATE REMOVED)

=> d l7 1-4 cbib abs

L7 ANSWER 1 OF 4 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. ON STN

2003210438 EMBASE Workshop overview: Approaches to the assessment of the allergenic potential of **food** from genetically **modified** crops. Ladics G.S.; Holsapple M.P.; Astwood J.D.; Kimber I.; Knippels L.M.J.; Helm R.M.; Dong W.. G.S. Ladics, DuPont-Haskell Laboratory, Elkton Road, Newark, DE 19714, United States. gregory.s.ladics@usa.dupont.com. Toxicological Sciences 73/1 (8-16) 1 May 2003.  
Refs: 35.

ISSN: 1096-6080. CODEN: TOSCF2. Pub. Country: United States. Language: English. Summary Language: English.

- AB There is a need to assess the safety of **foods** deriving from genetically **modified** (GM) crops, including the allergenic potential of novel gene products. Presently, there is no single in vitro or in vivo model that has been validated for the identification or characterization of potential **food allergens**. Instead, the evaluation focuses on risk factors such as source of the gene (i.e., allergenic vs. nonallergenic sources), physicochemical and genetic comparisons to known **allergens**, and exposure assessments. The purpose of this workshop was to gather together researchers working on various strategies for assessing protein allergenicity: (1) to describe the current state of knowledge and progress that has been made in the development and evaluation of appropriate testing strategies and (2) to identify critical issues that must now be addressed. This overview begins with a consideration of the current issues involved in assessing the allergenicity of GM **foods**. The second section presents information on in vitro models of digestibility, bioinformatics, and risk assessment in the context of clinical prevention and management of **food allergy**. Data on rodent models are presented in the next two sections. Finally, nonrodent models for assessing protein allergenicity are discussed. Collectively, these studies indicate that significant progress has been made in developing testing strategies. However, further efforts are needed to evaluate and validate the sensitivity, specificity, and reproducibility of many of these assays for determining the allergenicity potential of GM **foods**.

- L7 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2003 ACS on STN  
2002:736063 Document No. 137:277814 **Allergens** comprising deleted IgE-binding epitope and preserved T cell-activating ability for immunotherapy of **food allergies**. (Panacea Pharmaceuticals, USA).  
PCT Int. Appl. WO 2002074250 A2 20020926, 299 pp. DESIGNATED STATES: W: AE, AL, AM, AU, AZ, BB, BG, BY, CH, CZ, DK, EC, ES, FI, HU, ID, IN, KG, LC, LU, MA, MK, MW, RU, SD, SG, SI, SL, TN, UA, BY, KG, MD, RU, TJ, TM; RW: BE, BJ, CP, CH, CY, DE, ES, FI, GA, GB, GR, MR, NE, NL, PT, ES, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US9108 20020318. PRIORITY: US 2001-PV276822 20010316.

- AB It has been detd. that **allergens**, which are characterized by both humoral (IgE) and cellular (T-cell) binding sites, can be **modified** to be less allergenic by modifying the IgE binding sites. The IgE binding sites can be converted to non-IgE binding sites by altering as little as a single amino acid within the protein, preferably a hydrophobic residue towards the center of the IgE epitope, to eliminate IgE binding. Addnl. or alternatively a **modified allergen** with reduced IgE binding may be prepd. by disrupting one or more of the disulfide bonds that are present in the natural **allergen**. The disulfide bonds may be disrupted chem., e.g., by redn. and alkylation or by mutating one or more cysteine residues present in the primary amino acid sequence of the natural **allergen**. In certain embodiments, **modified allergens** are prepd. by both altering one or more linear IgE epitopes and disrupting one or more disulfide bonds of the natural **allergen**. In certain embodiments, the methods of the present invention allow **allergens** to be **modified** while retaining the ability of the protein to activate T-cells, and, in some embodiments by not significantly altering or decreasing IgG binding capacity. The Examples provided herein use peanut **allergens** to illustrate applications of the invention.

- L7 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2003 ACS on STN  
2001:762863 Document No. 135:317456 Synergistic improvements to polynucleotide vaccines. Raz, Eyal; Takabayashi, Kenji; Nguyen, Minh-Duc (The Regents of the University of California, USA). PCT Int. Appl. WO 2001076642 A1 20011018, 64 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG,

KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIIXXD2. APPLICATION: WO 2001-US11290 20010406. PRIORITY: US 2000-PV195890 20000407.

AB The invention features a polynucleotide vaccine **modified** to enhance expression of the encoded antigen in host cells. The polynucleotide vaccine comprises an antigen-encoding nucleic acid sequence derived from a non-host species of a first phylum or first kingdom, wherein the native signal sequence of the antigen coding sequence is deleted and, optionally, replaced with a signal sequence of a polypeptide of a second phylum or a second kingdom that is functional in the host to be immunized (e.g., a viral signal sequence with a plant antigen-encoding sequence). In one embodiment, the signal sequence is a hemagglutinin A (HA) signal sequence, and the antigen is an **allergen** (e.g., plant **allergen**) or from a pathogen (e.g., a bacterium, virus or parasite). The polynucleotide vaccine of the invention provides a synergistic effect with an immunostimulatory sequence (ISS) adjuvant to not only maintain, but to enhance, the immune response to the encoded antigen.

L7 ANSWER 4 OF 4 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STNDUPLICATE 1 2000231165 EMBASE Cloning of the minor **allergen** Api g 4 profilin from celery (*Apium graveolens*) and its cross-reactivity with birch pollen profilin Bet v 2. Scheurer S.; Wangorsch A.; Hausteiner D.; Vieths S. S. Vieths, Paul Ehrlich Institute, Department of Allergology, Paul Ehrlich Street 51-59, D-63225 Langen, Germany. Clinical and Experimental Allergy 30/7 (962-971) 2000. Refs: 40.

ISSN: 0954-7894. CODEN: CLEAEN. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB Background. Profilin is a panallergen that is recognized by IgE from about 20% of birch pollen- and plant **food**-allergic patients. A subgroup of celery-allergic patients shows IgE-reactivity with this minor **allergen**. To investigate the IgE-binding potential and cross-reactivity of celery profilin at the molecular level, this study was aimed at the cloning and immunological characterization of this **allergen**. Objectives. Cloning, expression and purification of profilin from celery tuber to characterize its immunological properties and its cross-reactivity with birch pollen profilin. Methods. Cloning of celery profilin was performed by polymerase chain reaction using degenerated primers and a 5'RACE method for the identification of the unknown 5'-end of the cDNA. Expression was carried out in *Escherichia coli* BL21 (DE3) using a **modified** vector pET30a. The recombinant profilin was purified by affinity chromatography on poly L-proline coupled to sepharose. Immunological characterization was performed by immunoblotting, EAST and IgE-inhibition experiments. Results. The coding region of the cDNA of celery profilin was identified as a 399-bp open reading frame, coding for a protein of 133 amino acids with a calculated molecular weight of 14.3 kDa. The deduced amino acid sequence of the corresponding protein showed high identity with other plant profilins (71-82%) recently described as **allergens**. Celery profilin was isolated as highly pure nonfusion protein. The IgE-reactivity of celery profilin was similar to that of natural protein. Seven of 17 celery-allergic patients tested presented specific IgE-antibodies to the recombinant protein tested by immunoblotting. Inhibition experiments showed high cross-reactivity of IgE with both profilins from celery and birch pollen. Moreover, the biological activity of recombinant celery profilin was demonstrated by a histamine release assay. Conclusions. Celery profilin is an important allergenic compound in celery and shows high homology to birch pollen profilin, Bet v 2. According to the revised IUIS **allergen** nomenclature, we suggest naming the celery profilin Api g 4. In addition to the cross-reacting major

**allergens** Api g 1 and Bet v 1, birch pollinosis and associated allergies to celery can therefore additionally be explained by the cross-reactivity between homologous profilins. Moreover, recombinant Api g 4 may be used for target-specific diagnosis and structural analyses.

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FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 14:56:44 ON 23 JUL 2003

L1 0 S MODIFIED FOOD ALLERGEN  
L2 109688 S ALLERGEN  
L3 11751 S L2 AND FOOD  
L4 431 S L3 AND MODIFIED  
L5 0 S L4 AND NUCLEOTIDE  
L6 5 S L4 AND NUCLEOTIDE  
L7 4 DUP REMOVE L6 (1 DUPLICATE REMOVED)

=> s l4 and IgE binding

L8 55 L4 AND IGE BINDING

=> dup remove l8

PROCESSING COMPLETED FOR L8

L9 26 DUP REMOVE L8 (29 DUPLICATES REMOVED)

=> d l9 1-26 cbib abs

L9 ANSWER 1 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
2003:239282 The Genuine Article (R) Number: 653AF. Engineered recombinant peanut protein and heat-killed *Listeria monocytogenes* coadministration protects against peanut-induced anaphylaxis in a murine model. Li X M (Reprint); Srivastava K; Huleatt J W; Bottomly K; Burks A W; Sampson H A. CUNY Mt Sinai Sch Med, Dept Pediat, 1 Gustave L Levy, New York, NY 10029 USA (Reprint); CUNY Mt Sinai Sch Med, Dept Pediat, New York, NY 10029 USA; Yale Univ, Sch Med, Immunol Sect, New Haven, CT 06520 USA; Univ Arkansas, Arkansas Childrens Hosp, Inst Res, Dept Pediat, Little Rock, AR 72205 USA. JOURNAL OF IMMUNOLOGY (15 MAR 2003) Vol. 170, No. 6, pp. 3289-3295. Publisher: AMER ASSOC IMMUNOLOGISTS. 9650 ROCKVILLE PIKE, BETHESDA, MD 20814 USA. ISSN: 0022-1767. Pub. country: USA. Language: English.  
\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Peanut allergy (PNA) is the major cause of fatal and near-fatal anaphylactic reactions to **foods**. Traditional immunotherapy using peanut (PN) protein is not an option for PNA therapy because of the high incidence of adverse reactions. We investigated the effects of s.c. injections of engineered (**modified**) recombinant PN proteins and heat-killed *Listeria monocytogenes* (HKLM) as an adjuvant on anaphylactic reactions in a mouse model of PN allergy. PN-allergic C3H/HeJ mice were treated s.c. with a mixture of the three major PN **allergens** and HKLM (**modified**) (mAra h 1-3 plus HKLM). The effects on anaphylactic reactions following PN challenge and the association with Ab levels and cytokine profiles were determined. Although all mice in the sham-treated groups exhibited anaphylactic symptoms with a median symptom score of 3, only 31% of mice in the mAra h 1-3 plus HKLM group developed mild anaphylaxis, with a low median symptom score of 0.5. Alterations in core body temperature, bronchial constriction, plasma histamine, and PN-specific IgE levels were all significantly reduced. This protective effect was markedly more potent than in the mAra h 1-3 protein alone-treated group. HKLM alone did not have any protective effect. Reduced IL-5 and IL-13, and increased IFN-gamma levels were observed only in splenocytes cultures from mAra h 1-3 plus HKLM-treated mice. These results show that immunotherapy with **modified** PN proteins and HKLM is effective for treating PN allergy in this model, and may be a potential approach for treating PNA.

L9 ANSWER 2 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STNDUPLICATE 1  
 2003:229053 The Genuine Article (R) Number: 654KW. Reduction of antigenicity and allergenicity of genetically **modified** egg white allergen, ovomucoid third domain. Mine Y (Reprint); Sasaki E; Zhang J W. Univ Guelph, Dept Food Sci, Guelph, ON N1G 2W1, Canada (Reprint). BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS (28 FEB 2003) Vol. 302, No. 1, pp. 133-137. Publisher: ACADEMIC PRESS INC ELSEVIER SCIENCE. 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA. ISSN: 0006-291X. Pub. country: Canada. Language: English.

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Ovomucoid (Gal d1) is a major **allergen** in hen egg white, consisting of three tandem domains. In this study, five genetically **modified** third domain (DIII) mutants, which were substituted single or double amino acids within its IgE and IgG epitopes were compared with those prepared and their antigenicity and allergenicity with native analogue using Western immunoblot and enzyme-linked immunosorbent assay. The replacement of phenylalanine at 37 (P37) position with methionine caused drastical loss of IgG and **IgE binding** activities of human sera derived from egg allergic patients as well as disruption of the alpha-helix structure which comprises a part of the IgG and IgE epitopes. Substituting glycine at 32 position in conjunction with P37 showed a synergistic effect of decreasing antigenicity. The present study indicated that glycine 32 and phenylalanine 37 have an important role on its antigenicity and allergenicity as well as structural integrity of ovomucoid DIII. (C) 2003 Elsevier Science (USA). All rights reserved.

L9 ANSWER 3 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
 2003:265700 The Genuine Article (R) Number: 654UF. Evaluation of potential allergenicity of genetically **modified** plants. Pasteau S (Reprint); Bannion G; Astwood J; Goodman R; Cockburn A. Monsanto Agr France SAS, Europarc Chene, 1 Rue Pierre Monod, F-69673 Bron, France (Reprint); Monsanto Agr France SAS, F-69673 Bron, France; Monsanto Co, Prod Safety Ctr, St Louis, MO USA; Monsanto Serv Int SA, Brussels, Belgium. REVUE FRANCAISE D ALLERGOLOGIE ET D IMMUNOLOGIE CLINIQUE (JAN 2003) Vol. 43, No. 1, pp. 24-30. Publisher: EDITIONS SCIENTIFIQUES MEDICALES ELSEVIER. 23 RUE LINOIS, 75724 PARIS CEDEX 15, FRANCE. ISSN: 0335-7457. Pub. country: France; USA; Belgium. Language: French.

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Establishing the safety of **foods** derived from genetically **modified** crops requires a multidisciplinary approach using methods adapted from the biochemical, nutritional, toxicological and immunological sciences. The core principle of the process has been articulated as substantial equivalence, which is a comparative evaluation. Concerning potential **allergens**, it is essential to evaluate newly introduced proteins and when necessary, to evaluate potential changes in endogenous **allergens**. The incidence of **food allergies** appears to be on the rise, particularly in developed countries. Since no cure is available for those afflicted with **food allergy**, disease management is achieved by avoidance of the offending **food**. As a result, significant weight in the assessment is given to the need for prevention, which in the context of safety assessment, means reducing the likelihood of transferring offending **allergens** from one **food** source to another. Genetic engineering of **food** crops should have little practical consequence for the occurrence, frequency and natural history of **food allergy** if this evaluation is robust. Essential aspects of the assessment for allergenicity of genetically **modified** crops are discussed in this article, (C) 2002 Editions scientifiques et medicales Elsevier SAS. All rights reserved.

L9 ANSWER 4 OF 26 CAPLUS COPYRIGHT 2003 ACS on STN  
 2002:736063 Document No. 137:277814 **Allergens** comprising deleted **IgE-binding** epitope and preserved T cell-activating ability for immunotherapy of **food allergies**. (Panacea

Pharmaceuticals, USA). PCT Int. Appl. WO 2002074250 A2 20020926, 299 pp. DESIGNATED STATES: W: AE, AL, AM, AU, AZ, BB, BG, BY, CH, CZ, DK, EC, ES, FI, HU, ID, IN, KG, LC, LU, MA, MK, MW, RU, SD, SG, SI, SL, TN, UA, BY, KG, MD, RU, TJ, TM; RW: BE, BJ, CF, CH, CY, DE, ES, FI, GA, GB, GR, MR, NE, NL, PT, ES, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US9108 20020318. PRIORITY: US 2001-PV276822 20010316.

AB It has been detd. that **allergens**, which are characterized by both humoral (IgE) and cellular (T-cell) binding sites, can be **modified** to be less allergenic by modifying the **IgE binding sites**. The **IgE binding sites** can be converted to non-**IgE binding sites** by altering as little as a single amino acid within the protein, preferably a hydrophobic residue towards the center of the IgE epitope, to eliminate **IgE binding**. Addnl. or alternatively a **modified allergen** with reduced **IgE binding** may be prepd. by disrupting one or more of the disulfide bonds that are present in the natural **allergen**. The disulfide bonds may be disrupted chem., e.g., by reduct. and alkylation or by mutating one or more cysteine residues present in the primary amino acid sequence of the natural **allergen**. In certain embodiments, **modified allergens** are prepd. by both altering one or more linear IgE epitopes and disrupting one or more disulfide bonds of the natural **allergen**. In certain embodiments, the methods of the present invention allow **allergens** to be **modified** while retaining the ability of the protein to activate T-cells, and, in some embodiments by not significantly altering or decreasing IgG binding capacity. The Examples provided herein use peanut **allergens** to illustrate applications of the invention.

L9 ANSWER 5 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
2003:230100 The Genuine Article (R) Number: 648ZQ. The allergic risk of transgenic **foods**: strategy for prevention. Moneret-Vautrin D A (Reprint). Cent Hosp, Serv Med Interne Immunol Clin & Allergol, 29 Ave Marechal Lattre De Tassigny, F-54035 Nancy, France (Reprint); Cent Hosp, Serv Med Interne Immunol Clin & Allergol, F-54035 Nancy, France. BULLETIN DE L ACADEMIE NATIONALE DE MEDECINE (15 MAR 2002) Vol. 186, No. 8, pp. 1391-1400. Publisher: ACADEMIE NATL DE MEDECINE. 16 RUE BONAPARTE, 75272 PARIS 06, FRANCE. ISSN: 0001-4079. Pub. country: France. Language: French. \*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Numerous **allergens** proceed from **foods**. The allergic risk of transgenic **foods** needs to be evaluated according recommendations from the Joint Expert Committee FAO/WHO. Potential issues are the risk of cross reactivity with existing **allergens**, the modification of allergenicity of the transgenic protein induced by a **modified metabolism** in the host, the **modified allergenicity** of the proteins of the transgenic plant, a potential neo-allergenicity of the transgenic protein, and the risk of dissemination through pollens inducing a respiratory sensitization then a cross **food allergy**. The algorithm includes three steps for evaluation: first the search for significant homology of the protein with **allergens** listed in **allergen databanks**, or the identity of a sequence of six aminoacids with known **allergens**, then a cross reactivity explored through the binding to IgEs from patients allergic to the source of the gene, or allergic to organisms of the same group or botanical family, and finally the extent of the pepsine resistance. The risk of immunogenicity has to be studied with appropriate animal models. A post-marketing surveillance is recommended for monitoring of adverse effects. The structure of an Allergo-Vigilance Network, the tools for efficiency and the groups at higher risk will be discussed.

L9 ANSWER 6 OF 26 MEDLINE on STN DUPLICATE 2  
2002:308565 Document Number: 22048553. PubMed ID: 12052675. Clinical risk assessment of GM **foods**. Lack Gideon. (Department of Paediatric Allergy and Immunology, Imperial College at St Mary's Hospital, Praed Street, London W2 1NY, UK.. gideon.lack@st-marys.nhs.uk) . TOXICOLOGY

LETTERS, (2002 Feb 28) 127 (1-3) 337-40. Journal code: 7709027. ISSN: 0378-4274. Pub. country: Netherlands. Language: English.

AB The main concerns about adverse effects of genetically **modified (GM) foods** on health are the transfer of antibiotic resistance, toxicity and allergenicity. There are two issues from an allergic standpoint. First, the transfer of a known **allergen** may occur from a crop into a non-allergenic target crop. The second scenario is the creation of a neo-**allergen** where de novo sensitisation occurs in the population. The first scenario occurred in 1996 when the 2S albumen protein from Brazil nut was transferred into soy bean (N. Engl. J. Med. 334 (1996) 688). 2S albumen was found to be a major Brazil nut **allergen** and the newly expressed protein in transgenic soy retained its allergenicity. Patients allergic to Brazil nuts and not to soy bean now showed an IgE mediated response towards GM soy bean. We argue that it is possible to prevent such occurrences by doing **IgE -binding** studies and taking into account physico-chemical characteristics of proteins and referring to known **allergen** databases. The second possible scenario of de novo sensitisation does not easily lend itself to risk assessment. We compare GM technology to traditional plant breeding and **food** processing methods. There is no evidence that the technology used for the production of GM **foods** poses an allergic threat per se compared to other methodologies widely accepted in the **food** industry. We need to proceed cautiously in the future, assessing individual GM **foods** on the basis of their individual merits and risks prior to introducing them into the market.

L9 ANSWER 7 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
2002:790685 The Genuine Article (R) Number: 596PQ. Bioinformatic methods for allergenicity assessment using a comprehensive **allergen** database  
Hileman R E; Silvanovich A; Goodman R E; Rice E A; Hollerschak G;  
Astwood J D; Hefle S L (Reprint). Univ Nebraska, Food Allergy Res &  
Resource Program, 255 Food Ind Bldg, Lincoln, NE 68583 USA (Reprint); Univ  
Nebraska, Food Allergy Res & Resource Program, Lincoln, NE 68583 USA;  
Monsanto Co, Prod Safety Ctr, St Louis, MO USA. INTERNATIONAL ARCHIVES OF  
ALLERGY AND IMMUNOLOGY (AUG 2002) Vol. 128, No. 4, pp. 280-291. Publisher:  
KARGER. ALLSCHWILERSTRASSE 10, CH-4009 BASEL, SWITZERLAND. ISSN: 1018-2438  
. Pub. country: USA. Language: English.

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*  
AB Background: A principal aim of the safety assessment of genetically **modified** crops is to prevent the introduction of known or clinically cross-reactive **allergens**. Current bioinformatic tools and a database of **allergens** and gliadins were tested for the ability to identify potential **allergens** by analyzing 6 *Bacillus thuringiensis* insecticidal proteins, 3 common non-allergenic **food** proteins and 50 randomly selected corn (*Zea mays*) proteins. Methods: Protein sequences were compared to **allergens** using the FASTA algorithm and by searching for matches of 6, 7 or 8 contiguous identical amino acids. Results: No significant sequence similarities or matches of 8 contiguous amino acids were found with the *B. thuringiensis* or **food** proteins. Surprisingly, 41 of 50 corn proteins matched at least one **allergen** with 6 contiguous identical amino acids. Only 7 of 50 corn proteins matched an **allergen** with 8 contiguous identical amino acids. When assessed for overall structural similarity to **allergens**, these 7 plus 2 additional corn proteins shared greater than or equal to 35% identity in an overlap of greater than or equal to 80 amino acids, but only 6 of the 7 were similar across the length of the protein, or shared >50% identity to an **allergen**. Conclusions: An evaluation of a protein by the FASTA algorithm is the most predictive of a clinically relevant cross-reactive **allergen**. An additional search for matches of 8 amino acids may provide an added margin of safety when assessing the potential allergenicity of a protein, but a search with a 6-amino-acid window produces many random, irrelevant matches. Copyright (C) 2002 S Karger AG, Basel.



L9 ANSWER 8 OF 26 MEDLINE on STN DUPLICATE 3  
 2002322193 Document Number: 22017607. PubMed ID: 12023205. Prediction of  
 allergenicity of gene-modified foods by serum-based  
 testing. Poulsen Lars K. (Laboratory of Medical Allergology, National  
 University Hospital, DK-2100 Copenhagen, Denmark.. lkpallgy@inet.uni2.dk)  
 . ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, (2002 May) 964 185-96. Ref:  
 41. Journal code: 7506858. ISSN: 0077-8923. Pub. country: United States.  
 Language: English.

AB On the basis of applying the IFBC/ILSI decision tree in a number of cases,  
 a refinement of the scheme is suggested. Large differences in allergenic  
 potential may be obtained by altering the route of administration of an  
**allergen**. Because an inhalation **allergen** can induce  
 symptoms at different threshold doses depending on whether it is  
 introduced via the inhalation or oral route, we propose that double-blind,  
 placebo-controlled food challenges be performed in all cases of  
 inhalation **allergens** being present in foods. Even  
 proteins never previously ingested may cause **IgE binding**  
 and elicit clinically relevant symptoms.

L9 ANSWER 9 OF 26 MEDLINE on STN DUPLICATE 4  
 2002095800 Document Number: 21657228. PubMed ID: 11799381. Linear IgE  
 epitope mapping of the English walnut (*Juglans regia*) major food  
**allergen**, Jug r 1. Robotham Jason M; Teuber Suzanne S; Sathe  
 Shridhar K; Roux Kenneth H. (Department of Biological Science and  
 Structural Biology Program, Florida State University, Tallahassee  
 32306-4370, USA.) JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, (2002 Jan)  
 109 (1) 143-9. Journal code: 1275002. ISSN: 0091-6749. Pub. country:  
 United States. Language: English.

AB BACKGROUND: Peanut and tree nut allergies can be life-threatening, and  
 they appear to be growing in prevalence. Jug r 1, a 2S albumin seed  
 storage protein, was previously characterized as a major English walnut  
**food allergen**. OBJECTIVE: We sought to identify the  
 linear **IgE-binding** epitopes of Jug r 1 and to  
 determine which, if any, amino acids are necessary for this binding to  
 occur. METHODS: Pools of sera from walnut-allergic patients and  
 overlapping peptides synthesized on an activated cellulose membrane were  
 used to screen for **IgE-binding** epitopes. Mutational  
 analysis of the immunodominant epitope was carried out through single and  
 multisite amino acid substitutions. Inhibition assays were performed  
 through use of affinity-purified IgE, soluble forms of the epitope  
 peptide, and the recombinant 2S albumin, rJug r 1. RESULTS: One  
 immunodominant linear epitope was identified. Amino acid mutations to the  
 epitope demonstrated that the residues RGEE, at positions 36 through 39,  
 were minimally required for **IgE binding**. Probing of  
 this epitope with sera from each of 20 patients revealed 15 of the sera to  
 be positive. Binding of patients' IgE to the epitope was inhibited with a  
 soluble form of the peptide; however, soluble peptide did not completely  
 inhibit the binding of IgE to the intact rJug r 1. CONCLUSION: One major  
 linear IgE-reactive epitope and its critical core amino acid residues have  
 been identified. Mutation of any of these core amino acids resulted in  
 loss of **IgE binding** to the epitope, and this points  
 toward the feasibility of reducing allergenicity in genetically  
**modified** walnuts. However, strong evidence for the existence of  
 conformational epitopes was also obtained.

L9 ANSWER 10 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
 2002:713737 The Genuine Article (R) Number: 585WR. Identifying and  
 eliminating **allergens**. Moneret-Vautrin D A (Reprint). Univ  
 Nancy 1, Serv Med Interne Immunol Clin & Allergol, Hop Cent, 29 Ave Lattre  
 de Tassigny, F-54035 Nancy, France (Reprint); Univ Nancy 1, Serv Med  
 Interne Immunol Clin & Allergol, Hop Cent, F-54035 Nancy, France.  
 OCL-OLEAGINEUX CORPS GRAS LIPIDES (MAR-JUN 2002) Vol. 9, No. 2-3, pp.  
 107-111. Publisher: JOHN LIBBEY EUROTEXT LTD. 127 AVE DE LA REPUBLIQUE,  
 92120 MONTROUGE, FRANCE. ISSN: 1258-8210. Pub. country: France. Language:  
 French.

- L9 ANSWER 11 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
 2002:196434 The Genuine Article (R) Number: 524XU. Protein allergenicity  
 assessment of **foods** produced through agricultural biotechnology.  
 Taylor S L (Reprint). Univ Nebraska, Food Allergy Res & Resource Program,  
 Lincoln, NE 68583 USA (Reprint). ANNUAL REVIEW OF PHARMACOLOGY AND  
 TOXICOLOGY (DEC 2002) Vol. 42, pp. 99-112. Publisher: ANNUAL REVIEWS. 4139  
 EL CAMINO WAY, PO BOX 10139, PALO ALTO, CA 94303-0139 USA. ISSN: 0362-1642  
 . Pub. country: USA. Language: English.  
 \*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*
- AB **Foods** produced through agricultural biotechnology are  
 reaching the consumer marketplace. These novel **foods** should be  
 assessed for their safety, including their potential allergenicity.  
 Agricultural biotechnology involves the introduction of novel proteins  
 into the **modified foods**, and proteins can be  
 allergenic. The potential allergenicity of the introduced proteins can be  
 evaluated by focusing on the source of the gene, the homology of the newly  
 introduced protein to known **allergens**, the reactivity of the  
 novel protein with IgE antibodies from the serum of individuals with known  
 allergies to the source of the transferred DNA or to materials that are  
 broadly related to the source of the transferred DNA, the resistance of  
 the novel protein to pepsin, and the immunoreactivity of the novel protein  
 in appropriate animal models. Additional factors, such as the level of  
 expression of the novel protein in the **modified food**  
 and expression in the edible portion of the **food**, may also yield  
 valuable insights. Applying such criteria provides a reasonable approach  
 to determining whether or not the novel protein is likely to become an  
**allergen**.
- L9 ANSWER 12 OF 26 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
 DUPLICATE 5
- 2003:87529 Document No.: PREV200300087529. Screening of transgenic proteins  
 expressed in transgenic **food** crops for the presence of short  
 amino acid sequences identical to potential, **IgE-binding**  
 linear epitopes of **allergens**. Kleter, Gijs A. (1); Peijnenburg,  
 Ad A. C. M.. (1) RIKILT Institute of Food Safety, NL 6700 AE, P.O. Box  
 230, Wageningen, Netherlands: g.a.kleter@rikilt.wag-ur.nl,  
 a.a.c.m.peijnenburg@rikilt.wag-ur.nl Netherlands. BMC Structural Biology,  
 (December 12 2002) Vol. 2, No. 8 Cited January 10, 2003, pp. No  
 Pagination. <http://www.biomedcentral.com/1472-6807>. online. ISSN:  
 1472-6807. Language: English.
- AB Background: Transgenic proteins expressed by genetically **modified**  
**food** crops are evaluated for their potential allergenic properties  
 prior to marketing, among others by identification of short identical  
 amino acid sequences that occur both in the transgenic protein and  
 allergenic proteins. A strategy is proposed, in which the positive  
 outcomes of the sequence comparison with a minimal length of six amino  
 acids are further screened for the presence of potential linear  
 IgE-epitopes. This double track approach involves the use of literature  
 data on IgE-epitopes and an antigenicity prediction algorithm. Results:  
 Thirty-three transgenic proteins have been screened for identities of at  
 least six contiguous amino acids shared with allergenic proteins.  
 Twenty-two transgenic proteins showed positive results of six- or  
 seven-contiguous amino acids length. Only a limited number of identical  
 stretches shared by transgenic proteins (papaya ringspot virus coat  
 protein, acetolactate synthase GH50, and glyphosate oxidoreductase) and  
 allergenic proteins could be identified as (part of) potential linear  
 epitopes. Conclusion: Many transgenic proteins have identical stretches of  
 six or seven amino acids in common with allergenic proteins. Most  
 identical stretches are likely to be false positives. As shown in this  
 study, identical stretches can be further screened for relevance by  
 comparison with linear **IgE-binding** epitopes described  
 in literature. In the absence of literature data on epitopes, antigenicity  
 prediction by computer aids to select potential antibody binding sites  
 that will need verification of **IgE binding** by sera

binding tests. Finally, the positive outcomes of this approach warrant further clinical testing for potential allergenicity.

- L9 ANSWER 13 OF 26 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
2002:268873 Document No.: PREV200200268873. Comparison of **allergens**  
in genetically **modified** soybean with conventional soybean. Park,  
Jae Hyun (1); Chung, Seung Tae (1); Kim, Jae Hee (1); Kim, Ji Young (1);  
Noh, Geun Woong; Kim, Dong Sup (1); Kim, Hyung Soo. (1) Department of  
Toxicology, National Institute of Toxicological Research, Korea Food and  
Drug Administration, 5 Nokbun-Dong, Eunpyung-Ku, Seoul, 122-704 South  
Korea. Yakhak Hoeji, (June, 2001) Vol. 45, No. 3, pp. 293-301. print.  
ISSN: 0513-4234. Language: Korean.
- AB Genetically **modified** organism (GMO) using recombinant DNA  
technique has been exponentially increased, however there are still  
arguments for the safety of **GM foods**. The objective of this  
research was to compare the **allergens** of GM soybean (Roundup  
ReadyTM) with conventional soybeans. Each soybean extracts were prepared  
as crude extracts, heated extracts, and as heated and simulated gastric  
fluid (SGF)-digested samples to characterize the stability of  
**allergens** to physicochemical treatment. Positive sera from 20  
soybean-sensitive patients and control sera from 5 normal subjects were  
used to identify the endogenous **allergens** in soybeans. Specific-  
**IgE binding** activities to each soybean preparations were  
evaluated by ELISA and immunoblot technique. In ELISA result, **IgE**  
**binding** activities of positive sera to soy crude extracts  
generally showed two fold higher mean value than those of control sera,  
however there was no significant difference between GM soybean and natural  
soybean varieties. Extracted proteins form each of the soybean  
preparations were separated with SDS-PAGE. The band pattern of GM soybean  
was very similar to those of natural soybean varieties. Immunoblots for  
the different soybeans revealed no differences in **IgE**-  
**binding** protein patterns, moreover, disclosed five prominent  
**IgE-binding** bands (75, 70, 50, 44 and 34 kDa) in crude  
extracts, four (75, 70, 44 and 34 kDa) in heated preparations, one (50  
kDa) in heated and SGF-digested preparations. These **IgE**  
**binding** bands were consistent with previously reported results on  
the soybean. These results indicate that GM soybean (Roundup ReadyTM) is  
no different from natural soybean in terms of its **allergen**.
- L9 ANSWER 14 OF 26 MEDLINE on STN DUPLICATE 6  
2002014512 Document Number: 21312429. PubMed ID: 11419707.  
Characterization and identification of **allergen** epitopes:  
recombinant peptide libraries and synthetic, overlapping peptides. Reese  
G; Ayuso R; Leong-Kee S M; Plante M J; Lehrer S B. (Tulane University  
Medical Center, Department of Medicine, New Orleans, LA 70112, USA..  
greese@tulane.edu) . JOURNAL OF CHROMATOGRAPHY. B, BIOMEDICAL SCIENCES AND  
APPLICATIONS, (2001 May 25) 756 (1-2) 157-63. Journal code: 9714109.  
ISSN: 1387-2273. Pub. country: Netherlands. Language: English.
- AB For the understanding of the relationship between protein structure and  
allergenicity, it is important to identify allergenic epitopes. Two  
methods to characterize primarily linear epitopes are compared using the  
major **allergen** from brown shrimp (*Penaeus aztecus*), Pen a 1, as  
an example. A recombinant peptide library was constructed and synthetic,  
overlapping peptides, spanning the entire Pen a 1 molecule, were  
synthesized and tested for specific IgE reactivity. Both methods  
identified **IgE-binding** of Pen a 1, however, the SPOTs  
procedure resulted in the identification of more epitopes of the major  
shrimp **allergen** Pen a 1 than the usage of the recombinant  
peptide library. For detection of specific IgE antibodies, the usage of  
125I-labeled detection antibody seems to be superior over enzyme-labeled  
anti IgE antibodies. The regeneration of SPOTs membranes is possible, but  
it is prudent to test regenerated membranes for residual activity. If a  
given **food allergen** contains significant linear  
epitopes, which seems to be true for stable major **allergens** such  
as those of peanut and shrimp the SPOTs system may be more advantageous

than the use of recombinant peptides libraries. However, if **allergens** are studied that contain more conformational epitopes, recombinant peptide libraries may help to identify the relevant epitopes. It has to be emphasized that no system for epitope identification will detect all epitopes and that the relevance of identified epitopes has to be confirmed with other methods such as inhibition studies, crystallographic analysis or the immunological evaluation of **modified whole allergens**.

- L9 ANSWER 15 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
 2001:451924 The Genuine Article (R) Number: 435PG. Characterization and identification of **allergen** epitopes: recombinant peptide libraries and synthetic, overlapping peptides. Reese G (Reprint); Ayuso R; Leong-Kee S M; Plante M J; Lehrer S B. Tulane Univ, Med Ctr, Dept Med, Allergy & Clin Immunol Sect, 1700 Perdido St, New Orleans, LA 70112 USA (Reprint); Tulane Univ, Med Ctr, Dept Med, Allergy & Clin Immunol Sect, New Orleans, LA 70112 USA. JOURNAL OF CHROMATOGRAPHY B (25 MAY 2001) Vol. 756, No. 1-2, pp. 157-163. Publisher: ELSEVIER SCIENCE BV. PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. ISSN: 0378-4347. Pub. country: USA. Language: English.  
 \*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*
- AB For the understanding of the relationship between protein structure and allergenicity, it is important to identify allergenic epitopes. Two methods to characterize primarily linear epitopes are compared using the major **allergen** from brown shrimp (*Penaeus aztecus*) Pen a 1, as an example. A recombinant peptide library was constructed and synthetic, overlapping peptides, spanning the entire Pen a 1 molecule, were synthesized and tested for specific IgE reactivity. Both methods identified **IgE-binding** of Pen a 1, however, the SPOTs procedure resulted in the identification of more epitopes of the major shrimp **allergen** Pen a 1 than the usage of the recombinant peptide library. For detection of specific IgE antibodies, the usage of I-125-labeled detection antibody seems to be superior over enzyme-labeled anti IgE antibodies. The regeneration of SPOTs membranes is possible, but it is prudent to test regenerated membranes for residual activity. If a given **food allergen** contains significant linear epitopes, which seems to be true for stable major **allergens** such as those of peanut and shrimp the SPOTs system may be more advantageous than the use of recombinant peptides libraries. However, if **allergens** are studied that contain more conformational epitopes, recombinant peptide libraries may help to identify the relevant epitopes. It has to be emphasized that no system for epitope identification will detect all epitopes and that the relevance of identified epitopes has to be confirmed with other methods such as inhibition studies, crystallographic analysis or the immunological evaluation of **modified whole allergens**. (C) 2001 Elsevier Science B.V. All rights reserved.
- L9 ANSWER 16 OF 26 MEDLINE on STN DUPLICATE 7  
 2001262411 Document Number: 21203243. PubMed ID: 11306930. Engineering, characterization and in vitro efficacy of the major peanut **allergens** for use in immunotherapy. Bannon G A; Cockrell G; Connaughton C; West C M; Helm R; Stanley J S; King N; Rabjohn P; Sampson H A; Burks A W. (Department of Biochemistry and Molecular Biology, Arkansas Children's Hospital Research Institute, Little Rock 72205, USA.. bannongarya@exchnage.uams.edu) . INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, (2001 Jan-Mar) 124 (1-3) 70-2. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.
- AB BACKGROUND: Numerous strategies have been proposed for the treatment of peanut allergies, but despite the steady advancement in our understanding of atopic immune responses and the increasing number of deaths each year from peanut anaphylaxis, there is still no safe, effective, specific therapy for the peanut-sensitive individual. Immunotherapy would be safer and more effective if the **allergens** could be altered to reduce their ability to initiate an allergic reaction without altering their

ability to desensitize the allergic patient. METHODS: The cDNA clones for three major peanut allergens, Ara h 1, Ara h 2, and Ara h 3, have been cloned and characterized. The **IgE-binding** epitopes of each of these allergens have been determined and amino acids critical to each epitope identified. Site-directed mutagenesis of the allergen cDNA clones, followed by recombinant production of the **modified allergen**, provided the reagents necessary to test our hypothesis that hypoallergenic proteins are effective immunotherapeutic reagents for treating peanut-sensitive patients. **Modified peanut allergens** were subjected to immunoblot analysis using peanut-positive patient sera IgE, T cell proliferation assays, and tested in a murine model of peanut anaphylaxis. RESULTS: In general, the **modified allergens** were poor competitors for binding of peanut-specific IgE when compared to their wild-type counterpart. The **modified allergens** demonstrated a greatly reduced **IgE-binding capacity** when individual patient serum IgE was compared to the binding capacity of the wild-type allergens. In addition, while there was considerable variability between patients, the **modified allergens** retained the ability to stimulate T cell proliferation. CONCLUSIONS: These **modified allergen** genes and proteins should provide a safe immunotherapeutic agent for the treatment of peanut allergy.

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L9 ANSWER 17 OF 26 CAPLUS COPYRIGHT 2003 ACS on STN  
 2000:666624 Document No. 133:251267 Immunostimulatory nucleic acids and antigens. Sosin, Howard B.; Caplan, Michael J. (Panacea Pharmaceuticals, LLC, USA). PCT Int. Appl. WO 2000054803 A2 20000921, 103 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2.  
 APPLICATION: WO 2000-US7213 20000316. PRIORITY: US 1999-PV124595 19990316; US 1999-PV125071 19990317.

AB The present invention provides methods and compns. for modulating an individual's immune response to antigens. It is an aspect of the present invention that allergic responses to antigens, which in some cases lead to asthma and even anaphylaxis, can be treated or prevented by administering compns. having immunostimulatory oligonucleotides having unmethylated CpG sequences. It is another aspect of the present invention that allergies to antigens, esp. one that result in asthma and anaphylaxis, can be treated or prevented by administering compns. contg. immunostimulatory oligonucleotides having unmethylated CpG dinucleotide sequences and further comprising antigen(s), fragments of the antigen, mixts. of fragments of the antigen, antigens **modified** to reduce Th2-type immune responses, and fragments of the antigen **modified** to reduce Th2-type immune responses. Cellular systems for studying immunostimulation by CpG contg. nucleic acids include in vivo, in vitro or ex vivo systems.

L9 ANSWER 18 OF 26 CAPLUS COPYRIGHT 2003 ACS on STN  
 2000:628260 Document No. 133:221613 Site-specific mutated **allergens** for decreased clinical reaction to allergy. Bannon, Gary A.; Burks, A. Wesley, Jr.; Sampson, Hugh A.; Sosin, Howard B.; King, Nina E.; Maleki, Soheila J.; Connaughton, Cathie; Kopper, Randall A.; Rabjohn, Patrick A.; Shin, David S.; Compadre, Cesar M. (The Board of Trustees of the University of Arkansas, USA; Mount Sinai School of Medicine of New York University). PCT Int. Appl. WO 2000052154 A2 20000908, 38 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK,

MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US5487 20000302. PRIORITY: US 1999-PV122566 19990302; US 1999-PV122960 19990303; US 1999-267719 19990311; US 2000-494096 20000128.

AB It has been detd. that **allergens**, which are characterized by both humoral (IgE) and cellular (T cell) binding sites, can be **modified** to be less allergenic by modifying the **IgE-binding sites**. The **IgE binding sites** can be converted to non-**IgE binding sites** by masking the site with a compd. that prevents **IgE binding** or by altering as little as a single amino acid within the protein, most typically a hydrophobic residue towards the center of the **IgE-binding epitope**, to eliminate **IgE binding**. The method allows the protein to be altered as minimally as possible, other than within the **IgE-binding sites**, while retaining the ability of the protein to activate T cells, and, in some embodiments by not significantly altering or decreasing IgG binding capacity. The examples use peanut **allergens** to demonstrate alteration of **IgE-binding sites**. The crit. amino acids within each of the **IgE-binding epitopes** of the peanut protein that are important to Ig binding were detd. Substitution of even a single amino acid within each of the epitopes led to loss of **IgE binding**. Although the epitopes shared no common amino acid sequence motif, the hydrophobic residues located in the center of the epitope appeared to be most crit. to **IgE binding**.

L9 ANSWER 19 OF 26 MEDLINE on STN DUPLICATE 8  
2000385116 Document Number: 20307538. PubMed ID: 10848918. Cloning of the minor **allergen** Api g 4 profilin from celery (Apium graveolens) and its cross-reactivity with birch pollen profilin Bet v 2. Scheurer S; Wangorsch A; Hausteiner D; Vieths S. (Paul Ehrlich Institute, Department of Allergology, Paul Ehrlich Street 51-59, D-63225 Langen, Germany.) CLINICAL AND EXPERIMENTAL ALLERGY, (2000 Jul) 30 (7) 962-71. Journal code: 8906443. ISSN: 0954-7894. Pub. country: ENGLAND: United Kingdom. Language: English.

AB BACKGROUND: Profilin is a panallergen that is recognized by IgE from about 20% of birch pollen- and plant food-allergic patients. A subgroup of celery-allergic patients shows IgE-reactivity with this minor **allergen**. To investigate the **IgE-binding** potential and cross-reactivity of celery profilin at the molecular level, this study was aimed at the cloning and immunological characterization of this **allergen**. OBJECTIVES: Cloning, expression and purification of profilin from celery tuber to characterize its immunological properties and its cross-reactivity with birch pollen profilin. METHODS: Cloning of celery profilin was performed by polymerase chain reaction using degenerated primers and a 5'RACE method for the identification of the unknown 5'-end of the cDNA. Expression was carried out in Escherichia coli BL21 (DE3) using a **modified** vector pET-30a. The recombinant profilin was purified by affinity chromatography on poly L-proline coupled to sepharose. Immunological characterization was performed by immunoblotting, EAST and IgE-inhibition experiments. RESULTS: The coding region of the cDNA of celery profilin was identified as a 399-bp open reading frame, coding for a protein of 133 amino acids with a calculated molecular weight of 14.3 kDa. The deduced amino acid sequence of the corresponding protein showed high identity with other plant profilins (71-82%) recently described as **allergens**. Celery profilin was isolated as highly pure nonfusion protein. The IgE-reactivity of celery profilin was similar to that of natural protein. Seven of 17 celery-allergic patients tested presented specific IgE-antibodies to the recombinant protein tested by immunoblotting. Inhibition experiments showed high cross-reactivity of IgE with both

profilins from celery and birch pollen. Moreover, the biological activity of recombinant celery profilin was demonstrated by a histamine release assay. CONCLUSIONS: Celery profilin is an important allergenic compound in celery and shows high homology to birch pollen profilin, Bet v 2. According to the revised IUIS allergen nomenclature, we suggest naming the celery profilin Api g 4. In addition to the cross-reacting major allergens Api g 1 and Bet v 1, birch pollinosis and associated allergies to celery can therefore additionally be explained by the cross-reactivity between homologous profilins. Moreover, recombinant Api g 4 may be used for target-specific diagnosis and structural analyses.

L9 ANSWER 20 OF 26 MEDLINE on STN DUPLICATE 9  
2002090936 Document Number: 20290936. PubMed ID: 10828721. Modulation of allergen-specific immune responses to the major shrimp allergen, tropomyosin, by specific targeting to scavenger receptors on macrophages. Rajagopal D; Ganesh K A; Subba Rao P V. (Department of Biochemistry, Indian Institute of Science, Bangalore, India. ) INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, (2000 Apr) 121 (4) 308-16. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB BACKGROUND: Tropomyosin from shrimp is the major cross-reacting crustacean food allergen. Earlier studies have led to the purification and immunochemical characterization of the major IgE binding epitopes of the allergen. Maleylated proteins are known to be specifically targeted to scavenger receptors on macrophage. Since antigens processed and presented by macrophages are known to elicit Th1 type of responses and allergic responses are characterized by polarization towards Th2 phenotype, the possibility of modulation of allergen-specific immune responses by targeting of tropomyosin to macrophage via scavenger receptor was explored. METHODS: The IgG and IgE binding potential of the native maleylated form of tropomyosin was carried out by ELISA and immunoblot. The ability of the native and maleylated form of allergen to induce in vitro proliferation of splenocytes from BALB/C mice immunized with both forms of allergen was tested. The in vitro production of IL-4 and IFN-gamma by splenocytes from mice immunized with the two forms of allergen was determined from culture supernatants. The in vivo production of serum IgG1 and IgG2a antibodies following immunization with native and modified allergens was monitored by ELISA. RESULTS: The maleylated form of tropomyosin was found to have reduced antigenicity and allergenicity as compared to its native counterpart. The modified allergen was, however, found to elicit a cellular response similar to native tropomyosin in vitro. Analysis of the cytokine profiles showed a modulation from an IL-4-dominant, proallergic, Th2 phenotype to an IFN-gamma-dominant, anti-allergic, Th1 phenotype that could also be correlated to a modulation in the in vivo antibody isotype. CONCLUSION: The results suggest the possible potential for modulating allergic responses in vivo by selective targeting to macrophages.  
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L9 ANSWER 21 OF 26 MEDLINE on STN DUPLICATE 10  
2000387444 Document Number: 20347073. PubMed ID: 10887324. Class I chitinases, the panallergens responsible for the latex-fruit syndrome, are induced by ethylene treatment and inactivated by heating. Sanchez-Monge R; Blanco C; Perales A D; Collada C; Carrillo T; Aragoncillo C; Salcedo G; Agronomos, Madrid, Spain. ) JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, (2000 Jul) 106 (1 Pt 1) 190-5. Journal code: 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB BACKGROUND: Class I chitinases have been identified as the major panallergens in fruits associated with the latex-fruit syndrome, such as avocado, banana, and chestnut. However, other plant foods containing these enzymes have not been related to this syndrome. OBJECTIVE: We sought out class I chitinases in the green bean, a legume

that is known to express chitinases but is not associated with latex allergy, and examined whether the content or allergenic activity of chitinases can be **modified** by physical or chemical treatments.

**METHODS:** **IgE-binding** proteins in untreated bean samples, as well as in ethylene- and heat-treated samples, were detected by using a pool of sera from patients with latex-fruit allergy. Putative **allergens** were purified by cation-exchange chromatography and characterized by N-terminal sequencing, enzymatic activity assays, immunodetection with sera and antichitinase antibodies, and immunoblot inhibition tests. Skin prick tests with untreated and heated purified **allergens** were also carried out. **RESULTS:** An **IgE-binding** protein of 32 kD that was also recognized by antichitinase antibodies was detected in green bean extracts. This reactive component was strongly induced by ethylene treatment. The protein, designated PvChI, was identified as a class I chitinase closely related to the major avocado **allergen** Prs a 1. Immunoblot inhibition assays demonstrated cross-reactivity between both **allergens**. Purified PvChI induced positive skin prick test responses in 7 of 8 patients with latex-fruit allergy. Heat treatment of both Prs a 1 and PvChI produced a full loss of their allergenic capacities both in vitro and in vivo. No **IgE-binding** component was detected in the white mature bean in which the main isolated 32-kD protein corresponded to a nonreactive phytohemagglutinin. **CONCLUSIONS:** Ethylene treatment induces the expression of plant class I chitinases. The allergenic activity of plant class I chitinases seems to be lost by heating. This fact could explain why plant **foods** containing these putative **allergens** that are consumed after cooking are not usually associated with the latex-fruit syndrome.

L9 ANSWER 22 OF 26 CAPLUS COPYRIGHT 2003 ACS ON STN  
 1999:630793 Document No. 132:150780 Pepsin-digested peanut contains T-cell epitopes but no IgE epitopes. Hong, Soo-Jong; Michael, J. Gabriel; Fehringer, Amy; Leung, Donald Y. M. (Division of Allergy-Immunology, The National Jewish Medical and Research, University of Colorado Health Sciences Center, Denver, CO, 80206, USA). Journal of Allergy and Clinical Immunology, 104(2, Pt. 1), 473-477 (English) 1999. CODEN: JACIBY. ISSN: 0091-6749. Publisher: Mosby, Inc..

AB Peanuts are a common cause of **food-induced** anaphylaxis and fatalities. Previous studies have demonstrated that rush immunotherapy to crude peanut ext. reduces clin. symptoms triggered by oral peanut challenges, but the immunotherapy was assocd. with an unacceptably high incidence of systemic allergic reactions. One approach to reduce the frequency of allergic reactions would be to use a **modified** peanut antigen with low allergenic properties. The authors sought to det. the immunol. characteristics of crude intact peanut ext. before and after pepsin digestion by using IgE immunoblotting and assessment of T-lymphocyte responses to intact and peptic digests of peanut exts. Western blot anal. of sera from 5 subjects with peanut allergy showed multiple IgE-reactive proteins in crude intact peanut ext. that were eliminated after pepsin treatment of the peanut ext. In contrast, pepsin-digested peanut induced significant T-cell proliferation responses (stimulation index = 30) in vitro in PBMCs from 7 subjects with peanut allergy, albeit at lower levels than that induced by intact peanut (stimulation index = 66). Furthermore, IFN-gamma. prodn. was induced by intact peanut and pepsin-digested peanut in a concn.-dependent manner. Importantly, T-cell lines generated in response to intact peanut also reacted to pepsin-digested peanut, indicating cross-reactive T-cell epitopes in intact and pepsin-digested peanut. These findings suggest that pepsin-digested peanut may be useful in peanut immunotherapy because pepsin digestion eliminates IgE reactivity but maintains T-cell reactivity.

L9 ANSWER 23 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI ON STN  
 1999:112329 The Genuine Article (R) Number: 162DN. Novel **food** products from genetically **modified** crop plants: methods and



future prospects. Dunwell J M (Reprint). UNIV READING, SCH PLANT SCI, READING, BERKS, ENGLAND (Reprint). INTERNATIONAL JOURNAL OF FOOD SCIENCE AND TECHNOLOGY (JUN 1998) Vol. 33, No. 3, pp. 205-213. Publisher: BLACKWELL SCIENCE LTD. P O BOX 88, OSNEY MEAD, OXFORD OX2 ONE, OXON, ENGLAND. ISSN: 0950-5423. Pub. country: ENGLAND. Language: English.  
\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

- AB Using a variety of in vitro techniques, it is now possible to isolate a selected gene sequence from any source and introduce it into any major crop plant. Millions of hectares of such genetically **modified** (GM) or transgenic plants are already being grown commercially, mostly in North America. To date, the most widely grown GM crops (soybean and maize) are those with **modified** agronomic traits (herbicide or insect tolerance); the products from these commodity crops are now included in a wide range of processed **foods**. This review describes the methods used to generate these GM crops and then discusses the range of **modified food** products that can be generated using this new technology. Such products include those with altered protein, starch or oil quality, as well as examples of improved micronutrient or vitamin content. Much of this work, particularly that aiming to develop **food** with specific health benefits, is still at the experimental stage, but there is no doubt that many GM foodstuffs, with an increasing variety of qualitative changes, will reach the market in the coming years. The rate at which such products are developed commercially depends to a large extent on the public reaction to a technology still poorly understood by most consumers.

- L9 ANSWER 24 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN 92:623081 The Genuine Article (R) Number: JU254. THE ANTIGENICITY AND ALLERGENICITY OF MICROPARTICULATED PROTEINS - SIMPLESSE(R). SAMPSON H A (Reprint); COOKE S. JOHNS HOPKINS UNIV, DEPT PEDIAT, DIV ALLERGY IMMUNOL, BALTIMORE, MD, 21218. CLINICAL AND EXPERIMENTAL ALLERGY (OCT 1992) Vol. 22, No. 10, pp. 963-969. ISSN: 0954-7894. Pub. country: USA. Language: ENGLISH.

- \*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*
- AB New technologies are allowing the food industry to develop products from standard **foods** which may not be recognized in its **modified** form by food allergic patients. One such product, Simplesse(R), has been formulated by microparticulation of egg white and/or cows' milk proteins and is used as a fat substitute in many fat-laden **foods**. The purpose of this study was to determine whether the process of microparticulation altered the allergenicity/antigenicity of egg white and cows' milk proteins compared to the starting materials.

Soluble protein fractions of Simplesse(R) and its respective starting materials were compared to egg white, cows' milk protein, an ultra-filtered egg white/condensed milk mixture, and/or a whey concentrate by SDS-polyacrylamide gel electrophoresis. In addition, sera from 16 patients with documented egg and/or cows' milk hypersensitivity and two controls who were not allergic to egg or milk were used to assess potential allergenicity/antigenicity of these products by immunoblot (Western blot) analysis. There were heterogeneous IgE and IgG binding patterns to the **food** fractions among these **food** allergic patients suggesting differing sensitivity patterns among the individuals tested. However, utilizing both SDS-PAGE and immunoblot analyses, the major **allergens** in the microparticulated products were the same as those found in the starting materials, egg and cows' milk. In addition, there was no evidence of 'novel' protein fractions in the Simplesse(R) test materials compared to the starting materials.

- L9 ANSWER 25 OF 26 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN 90059111 EMBASE Document No.: 1990059111. Epitope mapping of the major **allergen** from yellow mustard seeds, Sin a I. Menendez-Arias L.; Dominguez J.; Moneo I.; Rodriguez R.. Departamento de Bioquímica I, Facultad de Química, Universidad Complutense, 28040 Madrid, Spain. Molecular Immunology 27/2 (143-150) 1990.

ISSN: 0161-5890. CODEN: IMCHAZ. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB The antigenic sites on the major **allergen** from yellow mustard (*Sinapis alba* L.) seeds were studied using murine (BALB/c) monoclonal antibodies (mAb) and human IgE antibodies. Ten IgG1 (K) mAb from two fusions were analyzed. Competition and complementation studies performed with peroxidase labeled mAb reveal the existence of two main antigenic sites in Sin a I. All the described mAb failed to recognize the unordered carboxyamidomethylated polypeptide chains, with the single exception of 2B3, which binds the alkylated large chain. However, this mAb cannot react with the tetranitromethane-**modified** protein which retains the native conformation. This fact suggests that the only tyrosine of Sin a I, located in the large chain, may be part of a sequential epitope of the **allergen**. This chemical modification also alters the binding of the mAb 4A11 and 3F3 to the **allergen**, besides 2B3. The three mAb belong to the same complementation group. Specific **IgE binding** cannot be inhibited either by the large or small carboxyamidomethylated polypeptide chains, while the nitrated **allergen** shows a weaker inhibitory activity than the native Sin a I. 4A11, which is a tyrosine-dependent mAb, causes the greatest binding inhibition of the tested mAb on human IgE from atopic individuals, as determined from a reverse enzyme immunoassay, suggesting an important role played by tyrosine in the immunochemical recognition of Sin a I.

L9 ANSWER 26 OF 26 MEDLINE on STN  
87286808 Document Number: 87286808. PubMed ID: 3475557. Insoluble and soluble **allergens** from wheat grain and wheat dust: detection of **IgE binding** in inhalant and ingestion allergy. Walsh B J; Baldo B A; Bass D J; Clancy R; Musk A W; Wrigley C W. NEW ENGLAND AND REGIONAL ALLERGY PROCEEDINGS, (1987 Jan-Feb) 8 (1) 27-33. Journal code: 8306562. ISSN: 0742-2814. Pub. country: United States. Language: English.

AB The need for better in vitro testing for wheat allergy particularly involved correlating clinical evidence of Type I hypersensitivity with laboratory detection of specific IgE antibodies in serum. We report here an improvement in this relationship by the use of a **modified** method for RAST (Radioallergosorbent test), involving nitrocellulose as the solid phase and alkali (or ethanol) for extraction of **allergens** and treatment of discs. Serum IgE reactions with the full range of wheat grain and dust proteins were studied using this method and the results were related to wheat allergies due to flour ingestion and the inhalation of flour, pollen or grain dust.

=> s allergen  
L10 109688 ALLERGEN

=> a l10 and IgE binding  
A IS NOT A RECOGNIZED COMMAND  
The previous command name entered was not recognized by the system.  
For a list of commands available to you in the current file, enter  
"HELP COMMANDS" at an arrow prompt (=>).

=> s l1 and IgE binding  
L11 0 L1 AND IGE BINDING

=> s l10 and modified  
L12 2634 L10 AND MODIFIED

=> s l12 and IgE binding  
L13 189 L12 AND IGE BINDING

=> s l13 and reduced  
L14 51 L13 AND REDUCED

=> dup remove l14

PROCESSING COMPLETED FOR L14  
L15 24 DUP REMOVE L14 (27 DUPLICATES REMOVED)

=> d 115 1-24 cbib abs

L15 ANSWER 1 OF 24 CAPLUS COPYRIGHT 2003 ACS on STN  
2003:242373 Document No. 138:270285 Recombinant **allergen** with  
**reduced IgE binding** but undiminished T-cell  
antigenicity as immunotherapeutic of type I allergy. Deweerdt, Nicole;  
Singh, Mohan Bir; Bhalla, Prem L.; Swoboda, Ines (The University of  
Melbourne, Australia). PCT Int. Appl. WO 2003025009 A1 20030327, 66 pp.  
DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ,  
CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE,  
GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS,  
LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO,  
RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC,  
VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF,  
BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU,  
MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2.  
APPLICATION: WO 2002-AU1261 20020913. PRIORITY: AU 2001-7792 20010920.  
AB The present invention relates generally to reagents useful in the  
immunotherapeutic or immunoprophylactic treatment of allergic diseases.  
More particularly, the present invention provides **modified**  
**allergens** exhibiting **reduced IgE** interactivity including  
**reduced IgE** prodn.-stimulatory activity, while retaining T-cell  
antigenicity, which are useful in the immunomodulation of type I allergic  
disease conditions. The **allergens** comprise substitution,  
deletion or addn. mutants or variants of Lol p 5, Phl p 5, Pac p 5 and  
immunol. related **allergens**. The present invention further  
contemplates a method of immunomodulation of allergic diseases such as  
type I allergic disease conditions by the administration of  
**modified allergens** exhibiting **reduced IgE**  
interactivity while retaining T-cell antigenicity.

L15 ANSWER 2 OF 24 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
2003:239282 The Genuine Article (R) Number: 653AF. Engineered recombinant  
peanut protein and heat-killed Listeria monocytogenes coadministration  
protects against peanut-induced anaphylaxis in a murine model. Li X M  
(Reprint); Srivastava K; Huleatt J W; Bottomly K; Burks A W; Sampson H A.  
CUNY Mt Sinai Sch Med, Dept Pediat, 1 Gustave L Levy, New York, NY 10029  
USA (Reprint); CUNY Mt Sinai Sch Med, Dept Pediat, New York, NY 10029 USA;  
Yale Univ, Sch Med, Immunol Sect, New Haven, CT 06520 USA; Univ Arkansas,  
Arkansas Childrens Hosp, Inst Res, Dept Pediat, Little Rock, AR 72205 USA.  
JOURNAL OF IMMUNOLOGY (15 MAR 2003) Vol. 170, No. 6, pp. 3289-3295.  
Publisher: AMER ASSOC IMMUNOLOGISTS. 9650 ROCKVILLE PIKE, BETHESDA, MD  
20814 USA. ISSN: 0022-1767. Pub. country: USA. Language: English.  
\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Peanut allergy (PNA) is the major cause of fatal and near-fatal  
anaphylactic reactions to foods. Traditional immunotherapy using peanut  
(PN) protein is not an option for PNA therapy because of the high  
incidence of adverse reactions. We investigated the effects of s.c.  
injections of engineered (**modified**) recombinant PN proteins and  
heat-killed Listeria monocytogenes (HKLM) as an adjuvant on anaphylactic  
reactions in a mouse model of PN allergy. PN-allergic C3H/HeJ mice were  
treated s.c. with a mixture of the three major PN **allergens** and  
HKLM (**modified** (m)Ara h 1-3 plus HKLM). The effects on  
anaphylactic reactions following PN challenge and the association with Ab  
levels and cytokine profiles were determined. Although all mice in the  
sham-treated groups exhibited anaphylactic symptoms with a median symptom  
score of 3, only 31% of mice in the mAra h 1-3 plus HKLM group developed  
mild anaphylaxis, with a low median symptom score of 0.5. Alterations in  
core body temperature, bronchial constriction, plasma histamine, and  
PN-specific IgE levels were all significantly **reduced**. This  
protective effect was markedly more potent than in the mAra h 1-3 protein  
alone-treated group. HKLM alone did not have any protective effect.

**Reduced** IL-5 and IL-13, and increased IFN-gamma levels were observed only in splenocytes cultures from mArA h 1-3 plus HKLM-treated mice. These results show that immunotherapy with **modified** PN proteins and HKLM is effective for treating PN allergy in this model, and may be a potential approach for treating PNA.

L15 ANSWER 3 OF 24 MEDLINE on STN PubMed ID: 12823126. Analysis of the CD4+ T cell responses to house dust mite allergoid. Kalinski P; Lebre M C; Kramer D; De Jong E C; Van Schijndel J W P M; Kapsenberg M L. (Department of Cell Biology and Histology, Department of Dermatology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; Haarlems Allergenen Laboratorium, Haarlem, The Netherlands.) ALLERGY, (2003 Jul) 58 (7) 648-56. Journal code: 7804028. ISSN: 0105-4538. Pub. country: Denmark. Language: English.

AB BACKGROUND: **Modified allergen** extracts (allergoids) with **reduced IgE-binding** capacity are successfully used in immunotherapy of atopic allergy. Their **reduced** T-cell stimulatory capacity is less well studied and is a subject of the present study. METHODS: We compared the ability of native house dust mite extract (Dermatophagoides pteronyssinus; HDM) and the glutaraldehyde-**modified** allergoid (HDM-GA) to induce the proliferation and cytokine production by fresh PBMC and by DC-stimulated polyclonal Th cells and HDM-specific Th cell clones. RESULTS: Freshly isolated T cells showed a partially **reduced** responsiveness to HDM-GA, differentially pronounced in different donors. HDM-specific Th cell clones prepared from three donors showed either a complete loss of reactivity to HDM-GA, or completely preserved responsiveness. The frequency of nonreactive clones was donor-dependent (2/3, 3/10 and 1/10). GA modification of HDM did not interfere with the cytokine production profile of HDM-specific T cell clones. CONCLUSIONS: The **reduced** stimulatory potential of HDM-GA results mainly from a loss of certain Th cell epitopes, rather than impaired **allergen** uptake and presentation, or induction of suppressive factors. Varying frequencies of allergoid-nonreactive HDM-specific Th cells may result in differential responses of individual patients to immunotherapy.

L15 ANSWER 4 OF 24 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN 2003:117726 The Genuine Article (R) Number: 637WR. Hypoallergenic derivatives of major grass pollen **allergens** for allergy vaccination. Singh M B (Reprint); Bhalla P L. Univ Melbourne, Plant Mol Biol & Biotechnol Lab, Inst Land & Food Resources, Plant Mol Biol & Biotechnol Grp, Parkville, Vic, Australia (Reprint). IMMUNOLOGY AND CELL BIOLOGY (FEB 2003) Vol. 81, No. 1, pp. 86-91. Publisher: BLACKWELL PUBLISHING ASIA. 54 UNIVERSITY ST, P O BOX 378, CARLTON, VICTORIA 3053, AUSTRALIA. ISSN: 0818-9641. Pub. country: Australia. Language: English. \*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Grass pollen-induced hay-fever and allergic asthma represent a major health problem in industrialized countries. Whereas the symptoms of these allergic conditions can be controlled by pharmacotherapy, specific immunotherapy vaccination is the only causative approach towards the treatment of these type 1 allergies. Specific immunotherapy is based on administration of increasing amounts of the disease-causing **allergens** in the form of **allergen**-containing extracts. However, the extracts used for immunotherapy consist of allergenic and non-allergenic components and may induce severe anaphylactic side-effects upon therapeutic administration. With recent developments in molecular biology of pollen **allergens** it has become feasible to produce **modified** hypoallergenic derivatives of recombinant **allergens** with abrogated or greatly **reduced** likelihood of anaphylactic side-effects as compared to extract-based treatments. We have demonstrated this concept through reducing the anaphylactic potential of major rye grass pollen **allergens** by introducing a few point mutations which leave the overall structural fold of the molecule unaltered. These **modified** forms are expected to make

**allergen-specific immunotherapy** more widely used in the future.

L15 ANSWER 5 OF 24 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
2003:265705 The Genuine Article (R) Number: 654UF. Variants of  
**allergen** Phlp 5b and reduction of anaphylactogenic potential.  
Schramm G (Reprint); Kahlert H; Suck R; Weber B; Stuwe H T; Muller W D;  
Bufe A; Becker W M; Lepp U; Schlaak M W; Jager L; Cromwell O; Fiebig H.  
Zellulare Allergol Forschungszentrum Borstel, Parkallee 22, D-23845  
Borstel, Germany (Reprint); Zellulare Allergol Forschungszentrum Borstel,  
D-23845 Borstel, Germany; Allergopharma Joachim Ganzer KG, Reinbek,  
Germany; Univ Jena, D-6900 Jena, Germany. REVUE FRANCAISE D ALLERGOLOGIE  
ET D IMMUNOLOGIE CLINIQUE (JAN 2003) Vol. 43, No. 1, pp. 56-58. Publisher:  
EDITIONS SCIENTIFIQUES MEDICALES ELSEVIER. 23 RUE LINCOLN, 75724 PARIS  
CEDEX 15, FRANCE. ISSN: 0335-7457. Pub. country: Germany. Language:  
English.

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB One new approach to improved immunotherapy for type I allergy might be  
the use of **modified allergens** with **reduced**  
IgE reactivity but retained T cell reactivity. By site-directed  
mutagenesis outside the dominant T cell epitopes. We generated deletion  
Mutants of the grass pollen **allergen** Phl p 5b. Some of these  
variants revealed significantly **reduced** IgE reactivity and  
histamine releasing capacity compared to the wild-type **allergen**.  
Furthermore, in vivo skin prick tests showed that the variants had up to  
100 fold lower potency to induce cutaneous reactions than the wild-type  
**allergen**. On the other hand, T cell clones and T cell lines from  
grass pollen-allergic patients showed comparable proliferation after  
stimulation with these variants and wild-type **allergen**. Thus,  
variants of the **allergen** Phl p 5b with **reduced**  
anaphylactogenic potential but retained T cell reactivity could be  
valuable tools for improved **allergen-specific immunotherapy**. (C)  
2002 Editions scientifiques et medicales Elsevier SAS. All rights  
reserved.

L15 ANSWER 6 OF 24 CAPLUS COPYRIGHT 2003 ACS on STN  
2002:736063 Document No. 137:277814 **Allergens** comprising deleted  
**IgE-binding** epitope and preserved T cell-activating  
ability for immunotherapy of food allergies. (Panacea Pharmaceuticals,  
USA). PCT Int. Appl. WO 2002074250 A2 20020926, 299 pp. DESIGNATED  
STATES: W: AE, AL, AM, AU, AZ, BB, BG, BY, CH, CZ, DK, EC, ES, FI, HU,  
ID, IN, KG, LC, LU, MA, MK, MW, RU, SD, SG, SI, SL, TN, UA, BY, KG, MD,  
RU, TJ, TM; RW: BE, BJ, CF, CH, CY, DE, ES, FI, GA, GB, GR, MR, NE, NL,  
PT, ES, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO  
2000-US9108 20020318. PRIORITY: US 2001-PV276822 20010316.

AB It has been detd. that **allergens**, which are characterized by  
both humoral (IgE) and cellular (T-cell) binding sites, can be  
**modified** to be less allergenic by modifying the **IgE**  
**binding** sites. The **IgE binding** sites can be  
converted to non-**IgE binding** sites by altering as  
little as a single amino acid within the protein, preferably a hydrophobic  
residue towards the center of the IgE epitope, to eliminate **IgE**  
**binding**. Addnl. or alternatively a **modified**  
**allergen** with **reduced IgE binding**  
may be prep'd. by disrupting one or more of the disulfide bonds that are  
present in the natural **allergen**. The disulfide bonds may be  
disrupted chem., e.g., by redn. and alkylation or by mutating one or more  
cysteine residues present in the primary amino acid sequence of the  
natural **allergen**. In certain embodiments, **modified**  
**allergens** are prep'd. by both altering one or more linear IgE  
epitopes and disrupting one or more disulfide bonds of the natural  
**allergen**. In certain embodiments, the methods of the present  
invention allow **allergens** to be **modified** while  
retaining the ability of the protein to activate T-cells, and, in some  
embodiments by not significantly altering or decreasing IgG binding  
capacity. The Examples provided herein use peanut **allergens** to

illustrate applications of the invention.

L15 ANSWER 7 OF 24 MEDLINE on STN DUPLICATE 2  
2002614649 Document Number: 22258363. PubMed ID: 12371619.

**Allergen-specific T lymphocytes as targets for specific immunotherapy:** striking at the roots of type I allergy. Bohle Barbara. (Department of Pathophysiology, University of Vienna, Austria.. barbara.bohle@akh-wien.ac.at) . ARCHIVUM IMMUNOLOGIAE ET THERAPIAE EXPERIMENTALIS, (2002) 50 (4) 233-41. Ref: 80. Journal code: 0114365. ISSN: 0004-069X. Pub. country: Poland. Language: English.

AB In the past decades allergic diseases have tremendously increased and hypersensitivity reactions represent a growing health concern in industrialized countries. Despite various effective therapeutic options for the treatment of allergic diseases, only specific immunotherapy (SIT) has been shown to have effects on the underlying immunological mechanisms, namely functional changes at the level of T helper (Th) lymphocytes. It was found that **allergen-specific CD4+ Th2 lymphocytes** play a key role in the pathophysiology of atopic diseases. During successful SIT, the Th2-dominated immune response is **modified** towards a Th1 response, leading to a decline in **allergen-specific IgE** levels in the long term. In order to improve the efficacy and safety of SIT, novel approaches were developed targeting **allergen-specific Th2 lymphocytes** since specific inactivation or modulation towards Th1 cells could interfere with the disease process. In view of this aspect, this review will basically focus on two new, promising approaches to improve SIT: (1) the use of hypoallergenic proteins characterized by **reduced IgE-binding** capacities but retained T lymphocyte-activating properties and (2) oligodeoxynucleotides containing CpG motifs as an example of adjuvants which foster Th1 immune responses. Both approaches promise to be capable of adjusting the pathological Th2 immune response.

L15 ANSWER 8 OF 24 MEDLINE on STN DUPLICATE 3  
2002373866 Document Number: 22115009. PubMed ID: 12119498. Genetic engineering of **allergens:** future therapeutic products. Ferreira Fatima; Wallner Michael; Breiteneder Heimo; Hartl Arnulf; Thalhamer Josef; Ebner Christof. (Institute of Genetics, University of Salzburg, Salzburg, Austria.. fatima.ferreira@mh.sbg.ac.at) . INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, (2002 Jul) 128 (3) 171-8. Ref: 66. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB Genetic engineering of **allergens** for specific immunotherapy should aim at the production of **modified** molecules with **reduced IgE-binding** epitopes (hypoallergens), while preserving structural motifs necessary for T cell recognition (T cell epitopes) and for induction of IgG antibodies reactive with the natural **allergen** (blocking antibodies). Common approaches for engineering of hypoallergens usually require knowledge of T and B cell epitopes and involve changing specific base pairs (mutated gene), introduction of a new piece of DNA into the existing DNA molecule (chimeric or hybrid gene), and deletions (truncated gene or fragments). DNA family shuffling has the advantage that it does not require a priori knowledge of structural and functional properties for efficient generation of hypoallergens. The combination of the hypoallergen concept with the Th1-inducing genetic immunization approach might be an attractive alternative for protein-based immunotherapy. Copyright 2002 S. Karger AG, Basel

L15 ANSWER 9 OF 24 MEDLINE on STN DUPLICATE 4  
2002296618 Document Number: 22033045. PubMed ID: 12037397. Modification of peanut **allergen Ara h 3:** effects on **IgE binding** and T cell stimulation. Rabjohn Pat; West C Michael; Connaughton Cathie; Sampson Hugh A; Helm Ricki M; Burks A Wesley; Bannon Gary A. (Department of Biochemistry and Molecular Biology, Arkansas Children's Hospital Research Institute, University of Arkansas for Medical Sciences, Little Rock, AR 72202, USA. ) INTERNATIONAL ARCHIVES OF ALLERGY

AND IMMUNOLOGY, (2002 May) 128 (1) 15-23. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

- AB BACKGROUND: Peanut allergy is a major health concern due to the increased prevalence, potential severity, and chronicity of the reaction. The cDNA encoding a third peanut **allergen**, Ara h 3, has been previously cloned and characterized. Mutational analysis of the Ara h 3 **IgE** -**binding** epitopes with synthetic peptides revealed that single amino acid changes at critical residues could diminish **IgE** **binding**. METHODS: Specific oligonucleotides were used in polymerase chain reactions to modify the cDNA encoding Ara h 3 at critical **IgE** **binding** sites. Four point mutations were introduced into the Ara h 3 cDNA at codons encoding critical amino acids in epitopes 1, 2, 3 and 4. Recombinant **modified** proteins were used in SDS-PAGE/Western **IgE** immunoblot, SDS-PAGE/Western **IgE** immunoblot inhibition and T cell proliferation assays to determine the effects of these changes on in vitro clinical indicators of peanut hypersensitivity. RESULTS: Higher amounts of **modified** Ara h 3 were required to compete with the wild-type **allergen** for peanut-specific serum **IgE**. Immunoblot analysis with individual serum **IgE** from Ara-h-3-allergic patients showed that **IgE** **binding** to the **modified** protein decreased approximately 35-85% in comparison to **IgE** **binding** to wild-type Ara h 3. Also, the **modified** Ara h 3 retained the ability to stimulate T cell activation in PBMCs donated by Ara-h-3-allergic patients. CONCLUSIONS: The engineered hypoallergenic Ara h 3 variant displays two characteristics essential for recombinant **allergen** immunotherapy; it has a **reduced** binding capacity for serum **IgE** from peanut-hypersensitive patients and it can stimulate T-cell proliferation and activation. Copyright 2002 S. Karger AG, Basel

- L15 ANSWER 10 OF 24 MEDLINE on STN DUPLICATE 5  
2001420419 Document Number: 21361316. PubMed ID: 11468000. Allergic proteins are fragmented in low concentrations of sodium hypochlorite. Chen P; Eggleston P A. (Johns Hopkins University, 600 North Wolfe Street, Baltimore, MD 21287, USA. ) CLINICAL AND EXPERIMENTAL ALLERGY, (2001 Jul) 31 (7) 1086-93. Journal code: 8906443. ISSN: 0954-7894. Pub. country: England: United Kingdom. Language: English.

- AB BACKGROUND: To facilitate **allergen** removal from indoor environments, it would be helpful to have household cleaning products that **modified** allergenic activity. Because NaOCl dissolves proteins in high concentrations and is both capable of killing bacteria and viruses and inactivating viral antigens at somewhat lower concentrations, we explored its effects on Mus m 1 and other indoor **allergens**. OBJECTIVE: To examine the ability of NaOCl to reduce the allergenicity of Mus m 1 and other indoor **allergens**. METHODS: Using purified mouse urinary **allergen**, we examined the effect on protein measured by Coomassie protein assay and on Mus m 1 measured by ELISA. We also examined the effects using SDS/PAGE and Western blots probed with sheep anti-Mus m 1 and with allergic human serum. RESULTS: When NaOCl and Mus m 1 were combined in a molar ratio of 100 : 1, **IgE** **binding** to Mus m 1 on Western blot was significantly **reduced**. At higher NaOCl concentrations the protein appeared to fragment and eventually became undetectable. Fragmentation appeared to be random in that peptides of a wide range of apparent molecular weight were produced. The reaction was complete within 1-2 min at 0.1 : 10 ratios of greater than 200 : 1 and was optimal at pH 7.4. Immunological activity of other **allergens** (Fel d 1, Bla g 1, Der p 1) was decreased in vitro and dried **allergen** extracts were removed from surfaces. Adding an extraneous protein, BSA, to NaOCl:Mus m 1 solutions decreased the effect of NaOCl on the **allergen**. CONCLUSIONS: We concluded that NaOCl at concentrations commonly used in household products is capable of dramatically affecting allergenic protein.

- L15 ANSWER 11 OF 24 MEDLINE on STN  
2002228197 Document Number: 21961750. PubMed ID: 11964740. Can knowledge

of the molecular structure of **allergens** improve immunotherapy?.  
Pomes A; Chapman M D. (Asthma and Allergic Diseases Center, Department of  
Medicine, UVA Health System, Charlottesville, Virginia, USA..  
apomes@inbio.com) . Curr Opin Allergy Clin Immunol, (2001 Dec) 1 (6)  
549-54. Ref: 59. Journal code: 100936359. ISSN: 1528-4050. Pub. country:  
United States. Language: English.

AB Conventional immunotherapy may be associated with the development of  
adverse reactions, including anaphylaxis, due to the use of increasing  
doses of **allergen**. Standardization of extracts is necessary in  
order to assess the correct amount of **allergen** administered. In  
recent years, increased knowledge on the molecular structure of  
**allergens** has allowed the development of novel alternatives for  
immunotherapy. Initially, **allergens** were cloned and expressed  
as recombinant proteins in eukaryotic and prokaryotic systems.  
Crystallization of the purified proteins led to the elucidation of the  
tertiary structure of the **allergen**. Molecular biology  
techniques were used to construct **modified allergens**  
whose new **IgE binding** properties were studied. **IgE**  
antibody mapping combined with molecular modeling has allowed the  
recognition of **IgE binding** sites on the surface of the  
molecule. This information has been applied to the engineering of new  
**modified allergens**, with and without adjuvants, that  
retain immunogenicity but with **reduced allergenicity**. The use  
of these molecules for immunotherapy should allow the administration of  
greater doses of **allergen**, without the undesired side effects  
characteristic of conventional immunotherapy.

L15 ANSWER 12 OF 24 MEDLINE on STN DUPLICATE 6  
2001262411 Document Number: 21203243. PubMed ID: 11306930. Engineering,  
characterization and in vitro efficacy of the major peanut  
**allergens** for use in immunotherapy. Bannon G A; Cockrell G;  
Connaughton C; West C M; Helm R; Stanley J S; King N; Rabjohn P; Sampson H  
A; Burks A W. (Department of Biochemistry and Molecular Biology, Arkansas  
Children's Hospital Research Institute, Little Rock 72205, USA..  
bannongarya@exchnage.uams.edu) . INTERNATIONAL ARCHIVES OF ALLERGY AND  
IMMUNOLOGY, (2001 Jan-Mar) 124 (1-3) 70-2. Journal code: 9211652. ISSN:  
1018-2438. Pub. country: Switzerland. Language: English.

AB BACKGROUND: Numerous strategies have been proposed for the treatment of  
peanut allergies, but despite the steady advancement in our understanding  
of atopic immune responses and the increasing number of deaths each year  
from peanut anaphylaxis, there is still no safe, effective, specific  
therapy for the peanut-sensitive individual. Immunotherapy would be safer  
and more effective if the **allergens** could be altered to reduce  
their ability to initiate an allergic reaction without altering their  
ability to desensitize the allergic patient. METHODS: The cDNA clones for  
three major peanut **allergens**, Ara h 1, Ara h 2, and Ara h 3,  
have been cloned and characterized. The **IgE-binding**  
epitopes of each of these **allergens** have been determined and  
amino acids critical to each epitope identified. Site-directed  
mutagenesis of the **allergen** cDNA clones, followed by recombinant  
production of the **modified allergen**, provided the  
reagents necessary to test our hypothesis that hypoallergenic proteins are  
effective immunotherapeutic reagents for treating peanut-sensitive  
patients. **Modified peanut allergens** were subjected to  
immunoblot analysis using peanut-positive patient sera **IgE**, T cell  
proliferation assays, and tested in a murine model of peanut anaphylaxis.  
RESULTS: In general, the **modified allergens** were poor  
competitors for binding of peanut-specific **IgE** when compared to their  
wild-type counterpart. The **modified allergens**  
demonstrated a greatly **reduced IgE-binding**  
capacity when individual patient serum **IgE** was compared to the binding  
capacity of the wild-type **allergens**. In addition, while there  
was considerable variability between patients, the **modified**  
**allergens** retained the ability to stimulate T cell proliferation.  
CONCLUSIONS: These **modified allergen** genes and



proteins should provide a safe immunotherapeutic agent for the treatment of peanut allergy.  
Copyright 2001 S. Karger AG, Basel

- L15 ANSWER 13 OF 24 MEDLINE on STN PubMed ID: 11306924. Reduction in allergenicity of grass pollen by genetic engineering. Bhalla P L; Swoboda I; Singh M B. (Plant Molecular Biology and Biotechnology Laboratory, Institute of Land and Food Resources, University of Melbourne, Parkville, Australia.. p.bhalla@landfood.unimelb.edu.au) . INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, (2001 Jan-Mar) 124 (1-3) 51-4. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.
- AB BACKGROUND: Hay fever and allergic asthma triggered by grass pollen **allergens** affect approximately 20% of the population in cool temperate climates. Ryegrass is the dominant source of **allergens** due to its prodigious airborne pollen production. Lol p 5 or group 5 is among the most important and widespread grass pollen **allergen** because it reacts with IgE antibodies of more than 90% of grass pollen-allergic patients, contains most of the grass pollen-specific IgE epitopes and elicits strong biological responses. Significant efforts have been made in developing diagnostic and therapeutic reagents for designing new and more effective immunotherapeutic strategies for treatment of allergic diseases. An alternative approach to this problem could be to reduce the amount of **allergen** content in the source plant. METHODS: High velocity microprojectile bombardment was used to genetically engineer ryegrass. Antisense construct targeted to one of major **allergen**, Lol p 5, was introduced. The expression of antisense RNA was regulated by a pollen-specific promoter. Pollen was analysed for IgE reactivity. RESULTS: Analysis of proteins with **allergen**-specific monoclonal and polyclonal antibodies did not detect Lol p 5 in the transgenic pollen. The transgenic pollen showed remarkably **reduced** allergenicity as reflected by low IgE binding capacity of pollen extract as compared to control pollen. The transgenic ryegrass plants in which Lol p 5 gene expression is perturbed showed normal fertile pollen development. CONCLUSIONS: Our studies showed that it is possible to selectively 'switch off' **allergen** production in pollen of ryegrass demonstrating feasibility of genetic engineering of plants for **reduced** allergenicity.  
Copyright 2001 S. Karger AG, Basel
- L15 ANSWER 14 OF 24 MEDLINE on STN DUPLICATE 7 PubMed ID: 10925258. T cell reactivity with allergoids: influence of the type of APC. Kahlert H; Grage-Griebenow E; Stuwe H T; Cromwell O; Fiebig H. (Allergopharma Joachim Ganzer KG, Reinbek, Germany.. allergopharmakg@cs1.com) . JOURNAL OF IMMUNOLOGY, (2000 Aug 15) 165 (4) 1807-15. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.
- AB The use of allergoids for **allergen**-specific immunotherapy has been established for many years. The characteristic features of these chemically **modified allergens** are their strongly **reduced IgE binding** activity compared with the native form and the retained immunogenicity. T cell reactivity of chemically **modified allergens** is documented in animals, but in humans indirect evidence of reactivity has been concluded from the induction of **allergen**-specific IgG during immunotherapy. Direct evidence of T cell reactivity was obtained recently using isolated human T cells. To obtain further insight into the mechanism of action of allergoids, we compared the Ag-presenting capacity of different APC types, including DC and macrophages, generated from CD14+ precursor cells from the blood of grass pollen allergic subjects, autologous PBMC, and B cells. These APC were used in experiments together with Phl p 5-specific T cell clones under stimulation with grass pollen **allergen** extract, rPhl p 5b, and the respective allergoids. Using DC and macrophages, allergoids exhibited a pronounced and reproducible T

cell-stimulating capacity. Responses were superior to those with PBMC, and isolated B cells failed to present allergoids. Considerable IL-12 production was observed only when using the DC for Ag presentation of both **allergens** and allergoids. The amount of IL-10 in supernatants was dependent on the phenotype of the respective T cell clone. High IL-10 production was associated with suppressed IL-12 production from the DC in most cases. In conclusion, the reactivity of Th cells with allergoids is dependent on the type of the APC.

- L15 ANSWER 15 OF 24 MEDLINE on STN DUPLICATE 8  
2000290936 Document Number: 20290936. PubMed ID: 10828721. Modulation of **allergen**-specific immune responses to the major shrimp **allergen**, tropomyosin, by specific targeting to scavenger receptors on macrophages. Rajagopal D; Ganesh K A; Subba Rao P V. (Department of Biochemistry, Indian Institute of Science, Bangalore, India.) INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, (2000 Apr) 121 (4) 308-16. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.
- AB BACKGROUND: Tropomyosin from shrimp is the major cross-reacting crustacean food **allergen**. Earlier studies have led to the purification and immunochemical characterization of the major **IgE binding** epitopes of the **allergen**. Maleylated proteins are known to be specifically targeted to scavenger receptors on macrophage. Since antigens processed and presented by macrophages are known to elicit Th1 type of responses and allergic responses are characterized by polarization towards Th2 phenotype, the possibility of modulation of **allergen**-specific immune responses by targeting of tropomyosin to macrophage via scavenger receptor was explored. METHODS: The IgG and **IgE binding** potential of the native maleylated form of tropomyosin was carried out by ELISA and immunoblot. The ability of the native and maleylated form of **allergen** to induce in vitro proliferation of splenocytes from BALB/C mice immunized with both forms of **allergen** was tested. The in vitro production of IL-4 and IFN-gamma by splenocytes from mice immunized with the two forms of **allergen** was determined from culture supernatants. The in vivo production of serum IgG1 and IgG2a antibodies following immunization with native and **modified allergens** was monitored by ELISA. RESULTS: The maleylated form of tropomyosin was found to have **reduced** antigenicity and allergenicity as compared to its native counterpart. The **modified allergen** was, however, found to elicit a cellular response similar to native tropomyosin in vitro. Analysis of the cytokine profiles showed a modulation from an IL-4-dominant, proallergic, Th2 phenotype to an IFN-gamma-dominant, antiallergic, Th1 phenotype that could also be correlated to a modulation in the in vivo antibody isotype. CONCLUSION: The results suggest the possible potential for modulating allergic responses in vivo by selective targeting to macrophages. Copyright 2000 S. Karger AG, Basel

- L15 ANSWER 16 OF 24 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
2000:259241 The Genuine Article (R) Number: 297YX. Recombinant **allergens**: application to diagnostic and therapeutic perspectives. Pauli G (Reprint); Deviller P. HOP UNIV STRASBOURG, SERV PNEUMOL, BP 426, F-67091 STRASBOURG, FRANCE (Reprint). REVUE DES MALADIES RESPIRATOIRES (FEB 2000) Vol. 17, No. 1BIS, pp. 293-303. Publisher: MASSON EDITION. 120 BLVD SAINT-GERMAIN, 75280 PARIS 06, FRANCE. ISSN: 0761-8425. Pub. country: FRANCE. Language: French.

- \*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*
- AB Techniques of generic engineering applied to **allergens** have enabled the production of recombinant **allergens**. The validation of recombinant **allergens** implies that their immunological activity and their identity with natural **allergens** might be confirmed by in vitro and in vivo techniques carried out on a sufficiently large number of allergic subjects. Currently available results for the principal pneumoallergens are reported. Thus the work of validating recombinant **allergen** BeTvl has been confirmed by in vitro tests

and also by skin tests and nasal and bronchial provocation tests. The association of four recombinant **allergens** of phleole has enabled the detection in vitro of sensitisation to germinated pollens in 94.5% of patients. For mites the validity of group 2 recombinant **allergens** has been confirmed. A system enabling the expression of glycosylation of recombinant proteins was necessary to validate recombinant proteins in group 1 **allergens**. The recombinant **allergen** Blot5 is recognised as being effective in the detection of sensitization to *Blomia tropicalis*, a domestic **allergen** in sub tropical countries. The recombinant **allergens** Bla g 4 and Bla g 5 have been tested in vitro and in vivo and reactions were positive in nearly 50% of subjects sensitive to cockroaches. The recombinant Asp f 1 has been tested in subjects suffering from allergic bronchopulmonary aspergillosis and is positive in 60-85% of cases.

Some studies are available for recombinant **allergens** of certain animal antigens (Equ c 1, Bos d 2). The consequences of clarifying recombinant **allergens** are then analysed : obtaining better standardised **allergens** for diagnostic tests, studying the spectrum of specificities of IgE induced by an **allergen**, the quantification of specific IgE, a better approach to mixed allergies with the help of recombinant **allergens** of the principal mixed **allergens**. Some recent progress has led to the production of **modified** recombinant **allergens**: the synthesis of recombinant polypeptides corresponding to T epitopes, the production of isoform recombinant **allergens** with reduced allergenic activity, the production of recombinant **allergens** of **modified** allergenic molecules by directed mutations and the production of recombinant fragments of allergenic molecules. The use of **modified** recombinant **allergens** is a way of permitting research which would, in the future, lead to new modalities of specific immunotherapy.

L15 ANSWER 17 OF 24 CAPLUS COPYRIGHT 2003 ACS on STN  
 1999:495393 Document No. 131:143513 Methods and reagents for decreasing allergic reactions. Sosin, Howard; Bannon, Gary A.; Burks, A. Wesley, Jr.; Sampson, Hugh A. (University of Arkansas, USA; Mt. Sinai School of Medicine of the City University of New York). PCT Int. Appl. WO 9938978 A1 19990805, 46 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English).  
 CODEN: PIXXD2. APPLICATION: WO 1999-US2031 19990129. PRIORITY: US 1998-PV73283 19980131; US 1998-PV74590 19980213; US 1998-PV74624 19980213; US 1998-PV74633 19980213; US 1998-141220 19980827.

AB It has been detd. that **allergens**, which are characterized by both humoral (IgE) and cellular (T cell) binding sites, can be **modified** to be less allergenic by modifying the **IgE** binding sites. The **IgE** binding sites can be converted to non-**IgE** binding sites by masking the site with a compd. that prevents **IgE** binding or by altering as little as a single amino acid within the protein, most typically a hydrophobic residue towards the center of the **IgE**-binding epitope, to eliminate **IgE** binding. The method allows the protein to be altered as minimally as possible, other than within the **IgE**-binding sites, while retaining the ability of the protein to activate T cells, and, in some embodiments by not significantly altering or decreasing IgG binding capacity. The examples use peanut **allergens** to demonstrate alteration of **IgE** binding sites. The crit. amino acids within each of the **IgE** binding epitopes of the peanut protein that are important to Ig binding have been detd. Substitution of even a single amino acid within each of the epitopes led

to loss of **IgE binding**. Although the epitopes shared no common amino acid sequence motif, the hydrophobic residues located in the center of the epitope appeared to be most crit. to **IgE binding**.

L15 ANSWER 18 OF 24 MEDLINE on STN PubMed ID: 10500236.  
1999432292 Document Number: 99432292. Antisense-mediated silencing of a gene encoding a major ryegrass pollen allergen. Bhalla P L; Swoboda I; Singh M B. (Plant Molecular Biology and Biotechnology Laboratory, Institute of Land and Food Resources, University of Melbourne, Parkville, Victoria 3052, Australia.. p.bhalla@landfood.unimelb.edu.au) . PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1999 Sep 28) 96 (20) 11676-80. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Type 1 allergic reactions, such as hay fever and allergic asthma, triggered by grass pollen **allergens** are a global health problem that affects approximately 20% of the population in cool, temperate climates. Ryegrass is the dominant source of **allergens** because of its prodigious production of airborne pollen. Lol p 5 is the major allergenic protein of ryegrass pollen, judging from the fact that almost all of the individuals allergic to grass pollen show presence of serum IgE antibodies against this protein. Moreover, nearly two-thirds of the IgE reactivity of ryegrass pollen has been attributed to this protein. Therefore, it can be expected that down-regulation of Lol p 5 production can significantly reduce the allergic potential of ryegrass pollen. Here, we report down-regulation of Lol p 5 with an antisense construct targeted to the Lol p 5 gene in ryegrass. The expression of antisense RNA was regulated by a pollen-specific promoter. Immunoblot analysis of proteins with **allergen-specific** antibodies did not detect Lol p 5 in the transgenic pollen. The transgenic pollen showed remarkably **reduced allergenicity** as reflected by low **IgE-binding** capacity of pollen extract as compared with that of control pollen. The transgenic ryegrass plants in which Lol p 5 gene expression is perturbed showed normal fertile pollen development, indicating that genetic engineering of hypoallergenic grass plants is possible.

L15 ANSWER 19 OF 24 MEDLINE on STN DUPLICATE 9  
1999236624 Document Number: 99236624. PubMed ID: 10221435. Physicochemical and immunologic characterization of low-molecular-weight allergoids of Dactylis glomerata pollen proteins. Cirkovic T D; Bukilica M N; Gavrovic M D; Vujcic Z M; Petrovic S; Jankov R M. (Faculty of Chemistry, Belgrade, Yugoslavia.) ALLERGY, (1999 Feb) 54 (2) 128-34. Journal code: 7804028. ISSN: 0105-4538. Pub. country: Denmark. Language: English.

AB BACKGROUND: Orchard grass (Dactylis glomerata) pollen proteins were chemically **modified** by means of acid anhydrides (maleic and succinic anhydride) to obtain low-molecular-weight allergoids. Chemical modification in both cases led to the replacement of one positive charge (epsilon amino group of Lys) by one negative charge, yielding proteins with changed physicochemical properties in comparison to the native orchard grass-pollen proteins. METHODS: Physicochemical characterization of derivatives was done by gel chromatography, SDS-PAGE, and isoelectric focusing. To examine the **IgE-binding** properties of these derivatives, we carried out immunoblotting. To examine the ability of derivatives to induce IgG production, we immunized rabbits. Skin prick testing with the allergoids was performed on 15 individuals allergic to orchard grass pollens and on two healthy subjects. RESULTS: It was shown that the **modified** proteins retain their original molecular weights, but change pI to more acidic values. In the case of allergoids, a strong reduction in **IgE binding** was found. Immunization of rabbits with allergoids showed that the derivatives retain the ability to induce IgG production, and that the antisera obtained in such a way react to native (unmodified) extract. The ability of

derivatives to induce allergic reaction was significantly **reduced**. The patients (86.6%) included in our study exhibited less than 50% of native extract response. Among them, 53.3% had no response to one or both allergoids. CONCLUSIONS: These modification procedures yield allergoids with a **reduced** allergenic activity and preserved immunogenic potential suitable for use in immunotherapy.

L15 ANSWER 20 OF 24 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
1999:544997 The Genuine Article (R) Number: 214EK. Genetically engineered plant **allergens** with **reduced** anaphylactic activity. Singh M B (Reprint); deWeerd N; Bhalla P L. UNIV MELBOURNE, INST LAND & FOOD RESOURCES, PLANT MOL BIOL & BIOTECHNOL LAB, PARKVILLE, VIC 3052, AUSTRALIA (Reprint). INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY (JUN 1999) Vol. 119, No. 2, pp. 75-85. Publisher: KARGER. ALLSCHWILERSTRASSE 10, CH-4009 BASEL, SWITZERLAND. ISSN: 1018-2438. Pub. country: AUSTRALIA. Language: English.

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Allergy immunotherapy is based on the administration of increasing amounts of the disease-eliciting **allergens** in order to yield **allergen-specific** non-responsiveness. Success of this therapy is associated with modulation of the immune response to allergenic molecules at the level of T-helper cells and the induction of blocking antibodies. The extracts used for immunotherapy are highly heterogeneous preparations from natural sources and contain additional components, mostly proteins which are not well defined. Recombinant DNA technology offers novel tools for production of pure and well-characterised **allergens** for specific immunotherapy. However, high IgE reactivity of pure recombinant **allergens** is associated with an increased risk of potentially life-threatening anaphylactic reactions. A major improvement in **allergen-specific** immunotherapy may be achieved by using genetically engineered recombinant **allergens** with **reduced** anaphylactic activity. Recently the site-directed mutagenesis technique has been applied successfully to produce variants of major grass, birch and oilseed rape **allergens** with **reduced** IgE reactivity but retained T-cell reactivity. These **modified allergens** with **reduced** anaphylactic potential are novel candidates for safer and more effective **allergen-specific** immunotherapy.

L15 ANSWER 21 OF 24 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
1998:104721 The Genuine Article (R) Number: YT529. Diagnostic value of recombinant **allergens**. Pauli G (Reprint). HOP UNIV STRASBOURG, SERV PNEUMOL, BP 426, F-67091 STRASBOURG, FRANCE (Reprint). REVUE FRANCAISE D ALLERGOLOGIE ET D IMMUNOLOGIE CLINIQUE (JAN 1997) Vol. 37, No. 8, pp. 1093-1101. Publisher: EXPANSION SCI FRANCAISE. 31 BLVD LATOUR MAUBOURG, 75007 PARIS, FRANCE. ISSN: 0335-7457. Pub. country: FRANCE. Language: French.

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Genetic engineering techniques applied to **allergens** have allowed the production of recombinant **allergens**. Validation of recombinant **allergens** demands confirmation of their immunological activity and their identity with natural **allergens** by in vivo and in vitro techniques on a sufficiently large number of allergic subjects. The results currently available for the main respiratory **allergens** are reported. For example, the validity of the birch recombinant **allergen** Bet v 1 was confirmed by in vitro tests, but also by skin tests and nasal and bronchial challenge tests. The combination of four recombinant **allergens** of timothy allowed the in vitro detection of sensitization to Gramineae pollens in 94.5% of patients. The validity of up to 2 recombinant **allergens** has been confirmed for house dust mites. Systems of expression allowing glycosylation of recombinant proteins were necessary to validate group 1 recombinant **allergen** proteins. Recombinant **allergen** Blo t 5 has been tested in vitro and in vivo, and was found to be effective in the detection of sensitization to *Blomia tropicalis*, a

domestic **allergen** in subtropical countries. Only recombinant **allergen** Bla g 4 has been tested in vitro and in vivo, with positive reactions in almost 50% of subjects sensitized to cockroaches. Recombinant Asp f 1 was tested in subjects suffering from allergic bronchopulmonary aspergillosis, and was positive in 60 to 85% of cases. Studies are also available for recombinant **allergens** of phospholipase A2, the major **allergen** of bee venom. The consequences of the development of recombinant **allergens** are then analysed: better standardized **allergens** for diagnostic tests, study of the spectrum of specificities of the IgE induced by an **allergen**, quantification of specific IgE, better approach to cross-allergies using recombinant **allergens** of the main cross **allergens**. The application of recombinant **allergens** to basic research has led to production of **modified** recombinant **allergens**: synthesis of recombinant polypeptides corresponding to T epitopes, production of recombinant **allergens** isoforms with reduced allergenic activity, production of recombinant **allergens** of allergenic molecules **modified** by directed mutations. The use of these **modified** recombinant **allergens** is one line of research which, in the future, may lead to new modalities of specific desensitization. Other lines of research are also under investigation: inhibition of antigen-antibody reactions by the use of recombinant Fab-blocking molecules, and recombinant molecules of immunodominant haptens.

- L15 ANSWER 22 OF 24 MEDLINE on STN DUPLICATE 10  
9714840 Document Number: 9714840. PubMed ID: 9269506. Preseasonal specific immunotherapy with **modified** Phleum pratense allergenic extracts: tolerability and effects. Ricca V; Ciprandi G; Pesce G; Riccio A; Varese P; Pecora S; Canonica G W. (Servizio di Allergologia, Ospedale Koelliker de Missionari di Maria S.S. Consolata, Torino, Italia.) ALLERGOLOGIA ET IMMUNOPATHOLOGIA, (1997 Jul-Aug) 25 (4) 167-75. Journal code: 0370073. ISSN: 0301-0546. Pub. country: Spain. Language: English.
- AB The preparation of chemically **modified allergens**, with a reduced IgE binding capacity (responsible for side effects with traditional immunotherapy) but with the same or greater immunogenic activity, is one of the paths followed to obtain better results with specific immunotherapy (IT). The aim of the study was to evaluate the tolerability and effects of an extract Phleum pratense, **modified** with glutaraldehyde and absorbed on aluminium hydroxide, in controlling the seasonal symptomatology induced by grass pollen in a group of 10 monosensitized patients, compared to a group of 10 similar patients not treated with specific IT but with drugs alone. The monitoring parameters were: 1) Clinical: a) symptomatology after specific conjunctival provocation test (pre and post seasonal) and during the natural exposure to the **allergen** b) drug consumption. 2) Immunological (peripheral blood eosinophils, total and specific IgE, total specific IgG). 3) Cytological, before, during and after the pollen season. CONCLUSIONS: In subjects treated with specific IT a) both the overall symptomatology and the drug consumption resulted significantly reduced compared to the controls (p = 0.045); b) the phlogistic infiltrate showed a tendency to decrease during the pollen season; c) the peripheral blood eosinophils, total and specific IgE and IgG did not show any significant variation compared to the controls; d) no systemic reactions occurred and there were only two slight local reactions.

- L15 ANSWER 23 OF 24 MEDLINE on STN DUPLICATE 11  
97163754 Document Number: 97163754. PubMed ID: 9010561. Preseasonal specific immunotherapy with **modified** phleum pratense allergenic extracts: tolerability and effects. Vittorio R; Giorgio C; Giampaola G; Annamaria R; Paola V; Silvia P; Walter C G. (Servizio di allergologia, ospedale koelliker dei missionari di Maria S.S. Consolata, Torino, Italia.) ALLERGOLOGIA ET IMMUNOPATHOLOGIA, (1996 Nov-Dec) 24 (6) 255-62. Journal code: 0370073. ISSN: 0301-0546. Pub. country: Spain. Language: English.
- AB The preparation of chemically **modified allergens**, with

a **reduced IgE binding capacity** (responsible for side effects with traditional immunotherapy) but with the same or greater immunogenic activity, is one of the paths followed to obtain better results with specific immunotherapy (IT). The aim of the study was to evaluate the tolerability and effects of extracts of *Phleum pratense*, **modified** with glutaraldehyde and absorbed on aluminium hydroxide, in controlling the seasonal symptomatology induced by grass pollen in a group of 10 monosensitized patients, compared to a group of 10 similar patients not treated with specific IT but with drugs alone. The monitoring parameters were: 1) Clinical: a) symptomatology after specific conjunctival provocation test (pre and post seasonal) and during the natural exposure to the **allergen** b) drug consumption. 2) Immunological (peripheral blood eosinophils, total and specific IgE, total specific IgG). 3) Cytological, before, during and after the pollen season. Conclusions: in subjects treated with specific IT a) both the overall symptomatology and the drug consumption resulted significantly **reduced** compared to the controls ( $p = 0.045$ ); b) the phlogistic infiltrate showed a tendency to decrease during the pollen season; c) the peripheral blood eosinophils, total and specific IgE and IgG did not show any significant variation compared to the controls; d) no systemic reactions occurred and there were only two slight local reactions.

L15 ANSWER 24 OF 24 CAPLUS COPYRIGHT 2003 ACS on STN  
1992:632043 Document No. 117:232043 Improved preparation of cat dander **allergens** for immunotherapeutic purposes and uses therefor. Kuo, Mei Chang; Bond, Julian (Immologic Pharmaceutical Corp., USA). PCT Int. Appl. WO 9215613 A1 19920917, 36 pp. DESIGNATED STATES: W: AU, CA, JP; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1992-US1344 19920220. PRIORITY: US 1991-662193 19910228.

AB **Modified** human T-cell-reactive feline protein (TRFP), for use in place of cat dander ext. for desensitization treatment of individuals allergic to cats, has a substantially unaltered ability to stimulate T-cells from such individuals but a **reduced** ability to bind IgE from these individuals. Affinity-purified TRFP is **modified** by treatment with mild alkali to remove O-linked carbohydrate moieties; **modified** TRFP may also be produced by recombinant DNA technol. Thus, TRFP was treated with KOH at pH 12.5 and room temp. for 16 h to inhibit **IgE binding** totally in immunoblot expts. and to inhibit histamine-releasing activity 100-fold.

=> s allergoid  
L16 0 ALLERGOID

=> s allergoid  
L17 560 ALLERGOID

=> s l17 and food  
L18 2 L17 AND FOOD

=> dup remove l18  
PROCESSING COMPLETED FOR L18  
L19 2 DUP REMOVE L18 (0 DUPLICATES REMOVED)

=> d l19 1-2 cbib abs

L19 ANSWER 1 OF 2 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
93:387691 The Genuine Article (R) Number: LH102. POSITION PAPER - ALLERGEN STANDARDIZATION AND SKIN-TESTS. DREBORG S (Reprint); FREW A. ALLERGY (1993) Vol. 48, No. 14, Supp. S, pp. 49-82. ISSN: 0105-4538. Language: ENGLISH.

L19 ANSWER 2 OF 2 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN  
78120089 EMBASE Document No.: 1978120089. [Pre seasonal treatment of hayfever

with a pollen depot extract]. ZUR PRASAISONALEN BEHANDLUNG DES HEUSCHNUPFENS MIT EINEM DEPOT POLLENEXTRAKT (L TYROSIN ADSORBAT). Duengemann H.; Von Mayenburg J.; Rakoski J.. Dermatol. Klin., Techn. Univ., Munchen, Germany. Medizinische Klinik 72/24 (1071-1079) 1977. CODEN: MEKLA7. Pub. Country: Germany. Language: German. Summary Language: English.

AB 90 patients with 'hayfever' - i.e. pollen allergic diseases of the respiratory tract and the eyes - were treated in spring 1974 and 1975 with a tyrosin absorbed grass and rye depot extract with three injections before the pollen-season. Only 16 patients had an isolated grass-rye-pollen-allergy in skin test, all the other patients had additional extrinsic allergies. These results were proved by RAST. One third of the patients had bad side-effects (27 out of 90) during the treatment. 17 patients showed general reactions and ten patients very strong local reactions. We think that these side-effects were caused by contact with antigens (hairs of animals, **foods**, flowers of trees) during therapy and acute or chronic infections. The results of therapy were studied by a questionnaire one year after the first series of injections: there were 37 improved, 32 unchanged and/or worsened in pollen-season after treatment. Nevertheless the results encouraged us to continue treatment with the grass-rye-pollen-extract. It could be used as an 'initial treatment' in pollinosis patients with a wide spread allergy just before the beginning of the pollen season. It is necessary to fulfil all procedures of precaution which are in use in hyposensitization therapy to prevent stronger side-effects.

=> d his

(FILE 'HOME' ENTERED AT 14:56:33 ON 23 JUL 2003)

FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 14:56:44 ON 23 JUL 2003

```

L1      0 S MODIFIED FOOD ALLERGEN
L2      109688 S ALLERGEN
L3      11751 S L2 AND FOOD
L4      431 S L3 AND MODIFIED
L5      0 S L4 AND NUCLEOTIDE
L6      5 S L4 AND NUCLEOTIDE
L7      4 DUP REMOVE L6 (1 DUPLICATE REMOVED)
L8      55 S L4 AND IGE BINDING
L9      26 DUP REMOVE L8 (29 DUPLICATES REMOVED)
L10     109688 S ALLERGEN
L11     0 S L1 AND IGE BINDING
L12     2634 S L10 AND MODIFIED
L13     189 S L12 AND IGE BINDING
L14     51 S L13 AND REDUCED
L15     24 DUP REMOVE L14 (27 DUPLICATES REMOVED)
L16     0 S ALLERGROID
L17     560 S ALLERGROID
L18     2 S L17 AND FOOD
L19     2 DUP REMOVE L18 (0 DUPLICATES REMOVED)

```

=> s l4 and site directed mutagenesis  
L20 5 L4 AND SITE DIRECTED MUTAGENESIS

=> dup remove l14  
PROCESSING COMPLETED FOR L14  
L21 24 DUP REMOVE L14 (27 DUPLICATES REMOVED)

=> dup remove l20  
PROCESSING COMPLETED FOR L20  
L22 2 DUP REMOVE L20 (3 DUPLICATES REMOVED)

=> d l22 1-2 cbib abs



L22 ANSWER 1 OF 2 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
 2003:229053 The Genuine Article (R) Number: 654KW. Reduction of antigenicity  
 and allergenicity of genetically **modified** egg white  
**allergen**, ovomucoid third domain. Mine Y (Reprint); Sasaki E;  
 Zhang J W. Univ Guelph, Dept Food Sci, Guelph, ON N1G 2W1, Canada  
 (Reprint). BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS (28 FEB  
 2003) Vol. 302, No. 1, pp. 133-137. Publisher: ACADEMIC PRESS INC ELSEVIER  
 SCIENCE. 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA. ISSN: 0006-291X  
 . Pub. country: Canada. Language: English.

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Ovomucoid (Gal d1) is a major **allergen** in hen egg white,  
 consisting of three tandem domains. In this study, five genetically  
**modified** third domain (DIII) mutants, which were substituted  
 single or double amino acids within its IgE and IgG epitopes were compared  
 with those prepared and their antigenicity and allergenicity with native  
 analogue using Western immunoblot and enzyme-linked immunosorbent assay.  
 The replacement of phenylalanine at 37 (F37) position with methionine  
 caused drastical loss of IgG and IgE binding activities of human sera  
 derived from egg allergic patients as well as disruption of the  
 alpha-helix structure which comprises a part of the IgG and IgE epitopes.  
 Substituting glycine at 32 position in conjunction with F37 showed a  
 synergistic effect of decreasing antigenicity. The present study indicated  
 that glycine 32 and phenylalanine 37 have an important role on its  
 antigenicity and allergenicity as well as structural integrity of  
 ovomucoid DIII. (C) 2003 Elsevier Science (USA). All rights reserved.

L22 ANSWER 2 OF 2 MEDLINE on STN DUPLICATE 1  
 2001262411 Document Number: 21203243. PubMed ID: 11306930. Engineering,  
 characterization and in vitro efficacy of the major peanut  
**allergens** for use in immunotherapy. Bannon G A; Cockrell G;  
 Connaughton C; West C M; Helm R; Stanley J S; King N; Rabjohn P; Sampson H  
 A; Burks A W. (Department of Biochemistry and Molecular Biology, Arkansas  
 Children's Hospital Research Institute, Little Rock 72205, USA.  
 bannongarya@exchnage.uams.edu) . INTERNATIONAL ARCHIVES OF ALLERGY AND  
 IMMUNOLOGY, (2001 Jan-Mar) 124 (1-3) 70-2. Journal code: 9211652. ISSN:  
 1018-2438. Pub. country: Switzerland. Language: English.

AB BACKGROUND: Numerous strategies have been proposed for the treatment of  
 peanut allergies, but despite the steady advancement in our understanding  
 of atopic immune responses and the increasing number of deaths each year  
 from peanut anaphylaxis, there is still no safe, effective, specific  
 therapy for the peanut-sensitive individual. Immunotherapy would be safer  
 and more effective if the **allergens** could be altered to reduce  
 their ability to initiate an allergic reaction without altering their  
 ability to desensitize the allergic patient. METHODS: The cDNA clones for  
 three major peanut **allergens**, Ara h 1, Ara h 2, and Ara h 3,  
 have been cloned and characterized. The IgE-binding epitopes of each of  
 these **allergens** have been determined and amino acids critical to  
 each epitope identified. **Site-directed**  
**mutagenesis** of the **allergen** cDNA clones, followed by  
 recombinant production of the **modified allergen**,  
 provided the reagents necessary to test our hypothesis that hypoallergenic  
 proteins are effective immunotherapeutic reagents for treating  
 peanut-sensitive patients. **Modified peanut allergens**  
 were subjected to immunoblot analysis using peanut-positive patient sera  
 IgE, T cell proliferation assays, and tested in a murine model of peanut  
 anaphylaxis. RESULTS: In general, the **modified**  
**allergens** were poor competitors for binding of peanut-specific IgE  
 when compared to their wild-type counterpart. The **modified**  
**allergens** demonstrated a greatly reduced IgE-binding capacity when  
 individual patient serum IgE was compared to the binding capacity of the  
 wild-type **allergens**. In addition, while there was considerable  
 variability between patients, the **modified allergens**  
 retained the ability to stimulate T cell proliferation. CONCLUSIONS:  
 These **modified allergen** genes and proteins should

provide a safe immunotherapeutic agent for the treatment of peanut allergy.  
Copyright 2001 S. Karger AG, Basel

=> s 12 and site directed mutagenesis  
L23 216 L2 AND SITE DIRECTED MUTAGENESIS

=> s 123 and polynucleotide  
L24 0 L23 AND POLYNUCLEOTIDE

=> dup remove 123  
PROCESSING COMPLETED FOR L23  
L25 103 DUP REMOVE L23 (113 DUPLICATES REMOVED)

=> s 125 and food  
L26 9 L25 AND FOOD

=> dup remove 126  
PROCESSING COMPLETED FOR L26  
L27 9 DUP REMOVE L26 (0 DUPLICATES REMOVED)

=> d 127 1-9 cbib abs

L27 ANSWER 1 OF 9 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
2002:393843 The Genuine Article (R) Number: 549GP. Latex allergy: towards immunotherapy for health care workers. Sutherland M F; Suphioglu C; Rolland J M; O'Hehir R E (Reprint). Alfred Hosp, Dept Allergy Asthma & Clin Immunol, Commercial Rd, Prahran, Vic 3181, Australia (Reprint); Alfred Hosp, Dept Allergy Asthma & Clin Immunol, Prahran, Vic 3181, Australia; Alfred Hosp, Dept Pathol & Immunol, Prahran, Vic, Australia; Monash Univ, Clayton, Vic 3168, Australia; Cooperat Res Ctr Asthma, Sydney, NSW, Australia. CLINICAL AND EXPERIMENTAL ALLERGY (MAY 2002) Vol. 32, No. 5, pp. 667-673. Publisher: BLACKWELL PUBLISHING LTD. P O BOX 88, OSNEY MEAD, OXFORD OX2 ONE, OXON, ENGLAND. ISSN: 0954-7894. Pub. country: Australia. Language: English.

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*  
AB Latex allergy is an important allergic disease for which safe and readily available immunotherapy is currently lacking. Despite advances in latex glove technology and reduction in **allergen** content, there remains a core of severely allergic health care workers (HCW), particularly with concomitant **food** allergy, for whom **allergen** avoidance is insufficient. Current experience with immunotherapy using crude latex extracts has shown an unacceptable level of local and systemic side-effects. Latex **allergens** are extremely potent with a heightened capacity to cross-link effector cell-bound IgE and induce anaphylaxis. The predominant pattern of **allergen** reactivity among HCW is different from that among children with spina bifida, perhaps due to exposure to latex glove proteins, particularly via inhalation, rather than particle bound latex proteins present in urinary catheters. Recent studies using purified skin testing reagents have indicated that the most clinically important latex **allergens** amongst HCW are Hev b 5, 6 and 7. Elucidation of the molecular and cellular mechanisms of the immune response to these **allergens** is pivotal to facilitate the search for safer immunotherapy of latex allergy among HCW.

L27 ANSWER 2 OF 9 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
2002:512245 Document No.: PREV200200512245. Epitopic characterization of native bovine beta-lactoglobulin. Clement, Gilles (1); Boquet, Didier; Probert, Yveline; Bernard, Herve; Negroni, Luc; Chatel, Jean-Marc; Adel-Patient, Karine; Creminon, Christophe; Wal, Jean-Michel; Grassi, Jacques. (1) Laboratoire d'immunoallergie alimentaire, INRA-CEA, SPI Bat. 136, CE Saclay 91191, Gif/Yvette Cedex: gilles.clement@cea.fr France. Journal of Immunological Methods, (1 August, 2002) Vol. 266, No. 1-2, pp.

67-78. <http://www.elsevier.com/locate/jim>. print. ISSN: 0022-1759.  
Language: English.

- AB Two monoclonal antibodies (mAbs) (mAb 97 and mAb 117) selected from a panel of 52 mAbs directed against beta-lactoglobulin (BLG) have previously been used to develop a two-site enzyme immunometric assay (EIA) specific for the native form of the protein (J. Immunol. Methods 220 (1998) 25). In the present work, the conformational epitopes recognized by these two mAbs and by the 50 others have been studied. Firstly, an epitope map was drawn using a surface plasmon resonance (SPR) biosensor: the epitopes were organized in a circle of 11 overlapping and 1 nonoverlapping antigenic regions. Secondly, 55 site-directed BLGA mutants were prepared and tested by ELISA and competitive immunoassay to localize these 12 antigenic regions on the protein molecule. Among them, 20 mutants showed a 10- to 7500-fold decrease in relative affinity for the mAbs of one or several neighbouring regions: their circular dichroism (CD) spectra were identical to the spectrum of wild-type (WT) BLGA. At least one mutant was found for each of the 11 overlapping antigenic regions which circled the molecule and for the nonoverlapping one which was localized near the entrance of the calyx. The two mAbs initially chosen were each directed towards very conformation-dependent epitopes and were thus suitable for monitoring native BLG in food products and manufacturing processes. Other mAb pairs could be used to follow the fate of specific regions of the molecule during denaturation or proteolytic digestion.

- L27 ANSWER 3 OF 9 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
2003:66147 Document No.: PREV200300066147. An update of immunotherapy for specific allergies. Prescott, Susan L. (1); Jones, Catherine A.. (1) Department of Paediatrics, University of Western Australia, Princess Margaret Hospital, PO Box D184, Perth, WA, 6001, Australia: susanp@ichr.uwa.edu.au Australia. Current Drug Targets - Inflammation and Allergy, (March 2002, 2002) Vol. 1, No. 1, pp. 65-75. print. ISSN: 1568-010X. Language: English.

- L27 ANSWER 4 OF 9 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
2002:232880 The Genuine Article (R) Number: 528Q2. Recombinant allergens for immunotherapy. Chapman M D (Reprint); Smith A M; Vailes L D; Pomes A. INDOOR Biotechnol Inc, 1216 Harris St, Charlottesville, VA 22903 USA (Reprint); Univ Virginia, Dept Med, Asthma & Allergy Dis Ctr, Charlottesville, VA USA. ALLERGY AND ASTHMA PROCEEDINGS (JAN-FEB 2002) Vol. 23, No. 1, pp. 5-8. Publisher: OCEAN SIDE PUBLICATIONS INC. 95 PITMAN ST, PROVIDENCE, RI 02906 USA. ISSN: 1088-5412. Pub. country: USA. Language: English.

- \*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*  
AB Many of the problems associated with using natural allergenic products for allergy diagnosis and treatment can be overcome using genetically engineered recombinant allergens. Over the past 10 years, the most important allergens from mites, pollens, animal dander, insects, and foods have been cloned, sequenced, and expressed. Allergens have diverse biological functions (they may be enzymes, enzyme inhibitors, lipocalins, or structural proteins). High-level expression systems have been developed to produce recombinant allergens in bacteria, yeast, or insect cells. Recombinant allergens show comparable immunoglobulin E (IgE) antibody binding to natural allergens and show excellent reactivity on skin testing and in in vitro diagnostic tests. Recombinant allergens will enable innovative new strategies for allergen immunotherapy to be developed. These include peptide-based vaccines, engineered hypoallergens with reduced reactivity for IgE antibodies, nucleotide-conjugated vaccines that promote Th1 responses, and the possibility of developing prophylactic allergen vaccines.

- L27 ANSWER 5 OF 9 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
2001:451934 The Genuine Article (R) Number: 435PG. Recombinant food allergens. Lorenz A R; Scheurer S; Haustein D; Vieths S (Reprint) . Paul Ehrlich Inst, Dept Allergol, Paul Ehrlich Str 51-59, D-63225

Langen, Germany (Reprint); Paul Ehrlich Inst, Dept Allergol, D-63225  
Langen, Germany. JOURNAL OF CHROMATOGRAPHY B (25 MAY 2001) Vol. 756, No.  
1-2, pp. 255-279. Publisher: ELSEVIER SCIENCE BV. PO BOX 211, 1000 AE  
AMSTERDAM, NETHERLANDS. ISSN: 0378-4347. Pub. country: Germany. Language:  
English.

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

- AB Allergenic (glyco)proteins are the elicitors of **food**  
allergies and can cause acute severe hypersensitivity reactions.  
Recombinant **food allergens** are available in  
standardised quantity and constant quality. Therefore, they offer new  
perspectives to overcome current difficulties in the diagnosis, treatment  
and investigation of **food allergies**. This review summarises the  
expression strategies and characteristics of more than 40 recombinant  
**food allergens** that have been produced until today.  
Their IgE-binding properties are compared to those of their natural  
counterparts, in addition their application as diagnostic tools, the  
generation of hypoallergenic recombinant isoforms and mutants for  
therapeutic purposes, the determination of epitopes and cross-reactive  
structures are described. (C) 2001 Elsevier Science B.V. All rights  
reserved.

- L27 ANSWER 6 OF 9 MEDLINE on STN  
2001262411 Document Number: 21203243. PubMed ID: 11306930. Engineering,  
characterization and in vitro efficacy of the major peanut  
**allergens** for use in immunotherapy. Bannon G A; Cockrell G;  
Connaughton C; West C M; Helm R; Stanley J S; King N; Rabjohn P; Sampson H  
A; Burks A W. (Department of Biochemistry and Molecular Biology, Arkansas  
Children's Hospital Research Institute, Little Rock 72205, USA...  
bannongarya@exchngae.uams.edu) . INTERNATIONAL ARCHIVES OF ALLERGY AND  
IMMUNOLOGY, (2001 Jan-Mar) 124 (1-3) 70-2. Journal code: 9211652. ISSN:  
1018-2438. Pub. country: Switzerland. Language: English.

- AB BACKGROUND: Numerous strategies have been proposed for the treatment of  
peanut allergies, but despite the steady advancement in our understanding  
of atopic immune responses and the increasing number of deaths each year  
from peanut anaphylaxis, there is still no safe, effective, specific  
therapy for the peanut-sensitive individual. Immunotherapy would be safer  
and more effective if the **allergens** could be altered to reduce  
their ability to initiate an allergic reaction without altering their  
ability to desensitize the allergic patient. METHODS: The cDNA clones for  
three major peanut **allergens**, Ara h 1, Ara h 2, and Ara h 3,  
have been cloned and characterized. The IgE-binding epitopes of each of  
these **allergens** have been determined and amino acids critical to  
each epitope identified. **Site-directed**  
**mutagenesis** of the **allergen** cDNA clones, followed by  
recombinant production of the modified **allergen**, provided the  
reagents necessary to test our hypothesis that hypoallergenic proteins are  
effective immunotherapeutic reagents for treating peanut-sensitive  
patients. Modified peanut **allergens** were subjected to  
immunoblot analysis using peanut-positive patient sera IgE, T cell  
proliferation assays, and tested in a murine model of peanut anaphylaxis.  
RESULTS: In general, the modified **allergens** were poor  
competitors for binding of peanut-specific IgE when compared to their  
wild-type counterpart. The modified **allergens** demonstrated a  
greatly reduced IgE-binding capacity when individual patient serum IgE was  
compared to the binding capacity of the wild-type **allergens**. In  
addition, while there was considerable variability between patients, the  
modified **allergens** retained the ability to stimulate T cell  
proliferation. CONCLUSIONS: These modified **allergen** genes and  
proteins should provide a safe immunotherapeutic agent for the treatment  
of peanut allergy.  
Copyright 2001 S. Karger AG, Basel

- L27 ANSWER 7 OF 9 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
2000:137719 Document No.: PREV200000137719. Re-engineering patatin (Sol t 1)  
protein to eliminate IgE binding. Alibhai, Murtaza (1); Astwood, James

(1); Joyce, Elysia (1); Pershing, Jay (1); Sampson, Hugh; Purcell, John (1). (1) Monsanto Company, Sain Louis, MO USA. Journal of Allergy and Clinical Immunology., (Jan., 2000) Vol. 105, No. 1 part 2, pp. S79. Meeting Info.: 56th Annual Meeting of the American Academy of Allergy, Asthma and Immunology. San Diego, California, USA March 03-08, 2000 American Academy of Allergy, Asthma and Immunology. ISSN: 0091-6749. Language: English. Summary Language: English.

L27 ANSWER 8 OF 9 MEDLINE on STN  
1999432437 Document Number: 99432437. PubMed ID: 10502033. Pollen-related

**food allergy: cloning and immunological analysis of isoforms and mutants of Mal d 1, the major apple allergen, and Bet v 1, the major birch pollen allergen.** Son D Y; Scheurer S; Hoffmann A; Hausteind D; Vieths S. (Paul-Ehrlich-Institut, Department of Allergology, Paul-Ehrlich-Str. 51-59, D-63225 Langen, Germany. ) EUROPEAN JOURNAL OF NUTRITION, (1999 Aug) 38 (4) 201-15. Journal code: 100888704. ISSN: 1436-6207. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB BACKGROUND: Mal d 1, the major apple allergen, cross-reacts with IgE specific for the major birch pollen allergen, Bet v 1, and is responsible for birch pollen related food allergy to apple. Isoforms of Bet v 1 showing minor sequence variations display different binding capacity for specific IgE antibodies from allergic patients. Moreover, strain-dependent variation of allergenicity has been reported for apples. OBJECTIVE: To investigate the occurrence of strain-dependent isoforms of Mal d 1 which may differ in their allergenic potential, to obtain data on structures essential for binding of Mal d 1 to the antibody, and to gain insights into the structures responsible for its IgE cross-reactivity to Bet v 1. METHODS: The cDNA of Mal d 1 from various apple strains was amplified by a PCR strategy based on conserved regions of known Mal d 1-sequences, and sequenced. Two major isoforms of Mal d 1 were expressed as recombinant proteins and purified, as were different variants of the major birch pollen allergen, Bet v 1. Together with already existing recombinant birch pollen and apple allergens, these were subjected to allergenicity testing by IgE-immunoblotting, enzyme allergeo sorbent test and dose related mediator release. "Hot-spots" for IgE-reactivity were identified by site-directed mutagenesis. RESULTS: Twelve Mal d 1-clones were sequenced from 7 apple varieties and compared to 3 known Mal d 1 sequences. The clones were clustered into two groups, each showing a high degree of sequence identity to one of the known sequences and specific differences to the third sequence. No strain-specific sequences were identified. In contrast, apple strains with reported differences in allergenicity showed different expression levels of the major allergen. Immunologic testing of recombinant allergens revealed high IgE binding capacity of 2 major isoforms, named GD26 and GS29, with a slightly higher IgE binding capacity of GD26. Moreover, the allergenicity was similar to another r Mal d 1 reported in the literature, representing the isoform divergent from our clones. Mutational analysis of our Mal d 1 allergens identified serine in position 111 as essential for IgE binding. Allergenicity was almost depleted by changing this residue into a proline. Moreover, the corresponding serine residue, present in position 112 of Bet v 1, was in a similar manner crucial for the allergenicity of the birch pollen allergen. CONCLUSION: We conclude that divergent allergenicity of apple strains mainly depends on different expression levels of the major allergen. Introduction of a proline residue in position 111 of Mal d 1 and in position 112 of Bet v 1 led to a drastic reduction of allergenicity of both the pollen and the food allergen, obviously also removing the cross-reactive epitope. Mutants with reduced IgE-reactivity but maintained T-cell reactivity may represent new candidates for a safer specific immunotherapy with reduced side-effects.

L27 ANSWER 9 OF 9 MEDLINE on STN  
1999330275 Document Number: 99330275. PubMed ID: 10403481.

Cross-reactivity and epitope analysis of Pru a 1, the major cherry allergen. Scheurer S; Son D Y; Boehm M; Karamloo F; Franke S; Hoffmann A; Hausteil D; Vieths S. (Paul-Ehrlich-Institut, Department of Allergology, Langen, Germany.) MOLECULAR IMMUNOLOGY, (1999 Feb) 36 (3) 155-67. Journal code: 7905289. ISSN: 0161-5890. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A high percentage of birch pollen allergic patients experiences food hypersensitivity after ingestion of fresh fruits and vegetables. The cross-reactivity of the major allergens of sweet cherry (Pru a 1), apple (Mal d 1), pear (Pyr c 1), celery tuber (Api g 1) and carrot (Dau c 1) is due to structural similarities which are reflected by high amino acid sequence identities with Bet v 1a, the major birch pollen allergen. Apart from a strong cross-reactivity to Bet v 1a, IgE inhibition experiments with Mal d 1, Pru a 1 and Api g 1 demonstrated the presence of common and different epitopes among the tested food allergens. Secondary structure prediction of all investigated allergens indicated the presence of almost identical structural elements. In particular, the 'P-loop' region is a common domain of the pollen related food allergens and of pathogenesis related proteins. To identify the IgE binding epitopes, five overlapping recombinant Pru a 1 fragments representing the entire amino acid sequence with lengths of approximately 60-120 residues were investigated. Weak IgE binding capacity was measured exclusively with Pru a 1P4 (1-120) by immunoblotting, whereas none of the fragments showed allergenicity in the rat basophil leukaemia cell mediator release assay. Site-directed mutagenesis experiments with Pru a 1 revealed that amino acid S112 is critical for IgE binding of almost all patients sera tested. This reduced IgE binding was also observed with a single point mutant of Bet v 1a (S112P) and thus indicated serine 112 as an essential residue for preserving the structure of a cross-reactive IgE epitope. Moreover, two Pru a 1 mutants with an altered 'P-loop' region, showed a lowered IgE binding capacity for IgE from a subgroup of allergic patients. The investigation of essential features for preserving cross-reactive IgE-epitopes provides the structural basis for understanding the clinically observed cross-allergenicity between pollen and fruits. Moreover, non-anaphylactic allergen fragments or variants derived from the IgE-inducing pollen allergens may serve as useful tools for a new strategy of specific immunotherapy.

```
=> s milk
L28      382842 MILK

=> s l28 and mutagenesis
L29      513 L28 AND MUTAGENESIS

=> s l29 and site directed mutagenesis
L30      166 L29 AND SITE DIRECTED MUTAGENESIS

=> s l30 and IgE binding
L31      0 L30 AND IGE BINDING

=> s l30 and IgE
L32      0 L30 AND IGE

=> s l30 and non analytactic
L33      0 L30 AND NON ANALYTACTIC

=> s l30 and polynucleotide
L34      0 L30 AND POLYNUCLEOTIDE

=> dup remove l30
PROCESSING.COMPLETED FOR L30
L35      87 DUP REMOVE L30 (79 DUPLICATES REMOVED)
```

=> d 135 1-87 cdbib abs

L35 ANSWER 1 OF 87 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
2003:295668 The Genuine Article (R) Number: 660VU. Mcp1 encodes the  
molybdenum cofactor carrier protein in *Chlamydomonas reinhardtii* and  
participates in protection, binding, and storage functions of the cofactor  
. Ataya F S; Witte C P; Galvan A; Igeno M I; Fernandez E (Reprint). Univ  
Cordoba, Dept Bioquim & Biol Mol, Campus Rabanales, Edificio Severo Ochoa,  
E-14071 Cordoba, Spain (Reprint); Univ Cordoba, Dept Bioquim & Biol Mol,  
E-14071 Cordoba, Spain. JOURNAL OF BIOLOGICAL CHEMISTRY (28 MAR 2003) Vol.  
278, No. 13, pp. 10885-10890. Publisher: AMER SOC BIOCHEMISTRY MOLECULAR  
BIOLOGY INC. 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3996 USA. ISSN:  
0021-9258. Pub. country: Spain. Language: English.

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The molybdenum cofactor (Moco) is essential for the activity of all  
molybdoenzymes except nitrogenase. The cDNA for the Moco carrier protein  
(MocoCP) of *Chlamydomonas reinhardtii* has been cloned by reverse  
transcription PCR approaches with primers designed from microsequenced  
peptides of this protein. The *C. reinhardtii* MocoCP has been expressed in  
*Escherichia coli*. The recombinant protein has been purified to  
electrophoretic homogeneity and is found assembled into a homotetramer  
when Moco is not present under native conditions. Recombinant MocoCP has  
the same biochemical characteristics as MocoCP from *C. reinhardtii*, as it  
bound Moco from milk xanthine oxidase with high affinity,  
prevented Moco inactivation by oxygen, and transferred Moco efficiently to  
aponitrate reductase from the *Neurospora crassa* nit1 mutant. The genomic  
DNA sequence corresponding to the *Chlamydomonas* MocoCP gene, CrMcp1, also  
was isolated. This gene contained three introns in the coding region. The  
deduced amino acid sequence of CrMcp1 did not show a significant identity  
to functionally known proteins in the GenBank(TM) data base, although a  
significant conservation was found with bacterial proteins of unknown  
function. The results suggest that proteins having a Moco binding function  
probably exist in other organisms.

L35 ANSWER 2 OF 87 MEDLINE on STN DUPLICATE 1  
2003321982 Document Number: 22735845. PubMed ID: 12817083. Unique amino  
acids cluster for switching from the dehydrogenase to oxidase form of  
xanthine oxidoreductase. Kuwabara Yoshimitsu; Nishino Tomoko; Okamoto Ken;  
Matsumura Tomohiro; Eger Bryan T; Pai Emil F; Nishino Takeshi. (Department  
of Biochemistry and Molecular Biology, Nippon Medical School, 1-1-5  
Sendagi, Bunkyo-ku, Tokyo 113-8602, Japan. ) PROCEEDINGS OF THE NATIONAL  
ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (2003 Jul 8) 100 (14)  
8170-5. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United  
States. Language: English.

AB In mammals, xanthine oxidoreductase is synthesized as a dehydrogenase  
(XDH) but can be readily converted to its oxidase form (XO) either by  
proteolysis or modification of cysteine residues. The crystal structures  
of bovine milk XDH and XO demonstrated that atoms in the highly  
charged active-site loop (Gln-423-Lys-433) around the FAD cofactor  
underwent large displacements during the conversion, blocking the approach  
of the NAD+ substrate to FAD in the XO form as well as changing the  
electrostatic environment around FAD. Here we identify a unique cluster  
of amino acids that plays a dual role by forming the core of a relay  
system for the XDH/XO transition and by gating a solvent channel leading  
toward the FAD ring. A more detailed structural comparison and  
**site-directed mutagenesis** analysis experiments  
showed that Phe-549, Arg-335, Trp-336, and Arg-427 sit at the center of a  
relay system that transmits modifications of the linker peptide by  
cysteine oxidation or proteolytic cleavage to the active-site loop  
(Gln-423-Lys-433). The tight interactions of these residues are crucial  
in the stabilization of the XDH conformation and for keeping the solvent  
channel closed. Both oxidative and proteolytic generation of XO  
effectively leads to the removal of Phe-549 from the cluster causing a  
reorientation of the bulky side chain of Trp-336, which then in turn  
forces a dislocation of Arg-427, an amino acid located in the active-site

loop. The conformational change also opens the gate for the solvent channel, making it easier for oxygen to reach the reduced FAD in XO.

L35 ANSWER 3 OF 87 CAPLUS COPYRIGHT 2003 ACS on STN  
2003:329075 Document No. 139:18156 Expressive gene structures of proper and modified genes. Lipinski, Daniel; Szalata, Marlena; Kalak, Robert; Plawski, Andrzej; Nuc, Katarzyna; Kala, Marta; Juzwa, Wojciech; Slomska, Karolina; Gronek, Piotr; Jura, Jacek; Jura, Jolanta; Smorag, Zdzislaw; Pienkowski, Marek; Slomski, Ryszard (Zakl. Genetyki Czlowieka, Polska Akad. Nauk, Poznan, 60-479, Pol.). Biotechnologia (1), 48-73 (Polish) 2003. CODEN: BIECEV. ISSN: 0860-7796. Publisher: Instytut Chemii Bioorganicznej PAN.

AB The genetic construct WAP6xHisHGH contg. the gene encoding human growth hormone (hGH) and WAP promoter expressed in mammary gland of animals was prepd. The 5' end of the gene was modified by the addn. of sequence encoding six histidine residues and the sequence recognized by thrombin. In this way, the growth hormone can be easily purified by affinity chromatog. and cleaved with thrombin to an active form. In the next step, the genetic construct was introduced by microinjection into male pronuclei of fertilized oocytes. Transgene was detected in male rabbit of FO generation (no. 61). Twelve offspring of founder rabbit of generation F1 indicated transgene sequences. The presence of growth hormone was revealed in the samples of milk accumulated during the lactation of females of F1 generation. The genetic constructs contg. chain 1 and chain 2 of Feld1, and the major allergen produced by cat (Felis domesticus) were prepd. Both genes were inactivated by introduction into the sequences a pos. selectable marker aminoglycoside phosphotransferase (resistant to neomycin). Outside the region of homol. to Feld1 chain 1 and chain 2 genes, the neg. selectable marker - thymidine kinase gene was introduced. The genetic constructs pNTKFD1 and pNTKFD2 can be used in further expts. involving the inactivation of Feld1 genes in cat cells. Both genes were modified by **site-directed mutagenesis** using megastarter with Stop codon for premature termination of translation. The presence of mutation was confirmed by sequencing. The genetic constructs with human hGH gene and cat Feld1 gene were introduced into the bovine and cat fetal fibroblasts resp. in co-transfection with plasmid pGT-N29 contg. pos. selectable marker by lipofection, pptn. and electroinjection methods. After the selection, surviving cells were subjected to further mol. anal. The stable incorporation of the genetic constructs WAP6xHisHGH and WAPHGH into the genome were obsd.

L35 ANSWER 4 OF 87 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
2002:791871 The Genuine Article (R) Number: 595PB. **Site-directed mutagenesis** of the basic N-terminal cluster of pancreatic bile salt-dependent lipase - Functional significance. Aubert E; Sbarra V; Le Petit-Thevenin J; Valette A; Lombardo D (Reprint). Fac Med Timone, INSERM, U559, Unite Rech Physiopathol Cellules Epitheliales, 27 Blv Jean Moulin, F-13385 Marseille 05, France (Reprint); Fac Med Timone, INSERM, U559, Unite Rech Physiopathol Cellules Epitheliales, F-13385 Marseille 05, France. JOURNAL OF BIOLOGICAL CHEMISTRY (20 SEP 2002) Vol. 277, No. 38, pp. 34987-34996. Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3996 USA. ISSN: 0021-9258. Pub. country: France. Language: English.  
\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Previous studies have postulated the presence of a heparin-binding site on the bile salt-dependent lipase (BSDL), whereas two bile salt-binding sites regulate the enzyme activity. One of these sites may overlap with the tentative heparin-binding site at the level of an N-terminal basic cluster consisting of positive residues Lys(32), Lys(56), Lys(61), Lys(62), and Arg(63). The present study uses specific **site-directed mutagenesis** to determine the functional significance of this basic cluster. Mutations in this sequence resulted in recombinant enzymes that were able to bind to immobilized and to cell-associated heparin before moving throughout intestinal cells.



Recombinant BSDL was fully active on soluble substrate, but mutants were less active on micellar cholesteryl. oleate in comparison with the wild-type enzyme. Activation studies by primary (sodium taurocholate) and by secondary (sodium taurodeoxycholate) bile salts revealed that the activation of BSDL by sodium taurocholate at concentrations below the critical micellar concentration, and not that evoked by micellar bile salts, was affected by substitutions, suggesting that this N-terminal basic cluster likely represents the specific bile salt-binding site of BSDL. Substitutions also affected the activation of the enzyme promoted by anionic phospholipids, extending the function of this site to that of a cationic regulatory site susceptible to accommodate anionic ligands.

L35 ANSWER 5 OF 87 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. ON STN  
2002:468004 Document No.: PREV200200468004. Manipulating monomer-dimer

equilibrium of bovine beta-lactoglobulin by amino acid substitution. Sakurai, Kazumasa; Goto, Yuji (1). (1) Institute for Protein Research, Osaka University, Yamadaoka 3-2, Suita, Osaka, 565-0871: ygoto@protein.osaka-u.ac.jp Japan. Journal of Biological Chemistry, (July 12, 2002) Vol. 277, No. 28, pp. 25735-25740. <http://www.jbc.org/>. print. ISSN: 0021-9258. Language: English.

AB Bovine beta-lactoglobulin, a major protein in cow's milk composed of nine beta-strands (betaA-betaI) and one alpha-helix, exists as a dimer at neutral pH while it dissociates to a native monomer below pH 3.0. It is assumed that the intermolecular beta-sheet formed between I-strands and salt bridges at AB-loops play important roles in dimer formation. Several site-directed mutants in which intermolecular interactions stabilizing the dimer would be removed were expressed in the methylotrophic yeast *Pichia pastoris*, and their monomer-dimer equilibria were studied by analytical ultracentrifugation. Various I-strand mutants showed decreases in  $K_{\text{dimer}}$ , suggesting that the intermolecular beta-sheet is essential for dimer formation. By substituting either Asp33 or Arg40 on the AB-loop to oppositely charged residues (i.e. R40D, R40E, and D33R), a large decrease in  $K_{\text{dimer}}$  was observed probably because of the charge repulsion, which is consistent with the role of electrostatic attraction between Arg40 on one monomer and Asp33 on the other monomer in the wild-type dimer. However, when two of these mutants, R40D and D33R, were mixed, a heterodimer was formed by the electrostatic attraction between Arg33 and Asp40 of different molecules. These results suggested that protein-protein interactions of bovine beta-lactoglobulin can be manipulated by redesigning the residues on the interface without affecting global folding.

L35 ANSWER 6 OF 87 MEDLINE ON STN DUPLICATE 2  
2002:640127 Document Number: 22286543. PubMed ID: 12399502.

Characterization of the two-component abortive phage infection mechanism AbiI from *Lactococcus lactis*. Bouchard Julie D; Dion Eric; Bissonnette Frederic; Moineau Sylvain. (Department of Biochemistry and Microbiology, Faculte des Sciences et de Genie, Groupe de Recherche en Ecologie Buccale, Faculte de Medecine Dentaire, Universite Laval, Quebec, Canada G1K 7P4. ) JOURNAL OF BACTERIOLOGY, (2002 Nov) 184 (22) 6325-32. Journal code: 2985120R. ISSN: 0021-9193. Pub. country: United States. Language: English.

AB During the production of fermented dairy products, virulent bacteriophages infecting *Lactococcus lactis* can delay or stop the milk acidification process. A solution to this biological problem consists of introducing natural phage barriers into the strains used by the dairy industry. One such hurdle is called abortive infection (Abi) and causes premature cell death with no or little phage progeny. Here, we describe the isolation and characterization of a novel Abi mechanism encoded by plasmid pED1 from *L. lactis*. The system is composed of two constitutively cotranscribed genes encoding putative proteins of 127 and 213 amino acids, named AbiTi and AbiTii, respectively. **Site-directed mutagenesis** indicated that a hydrophobic region at the C-terminal extremity of AbiTi is essential to the antiphage phenotype. The AbiT system is effective against phages of the 936 and P335 species (efficiency of plaquing between  $10^{-5}$  and  $10^{-7}$ ) and causes a 20-fold reduction in

the efficiency to form centers of infection as well as a 10- to 12-fold reduction in the burst size. Its efficacy could be improved by raising the plasmid copy number, but changing the intrinsic ratio of AbiTi and AbiTiII did not greatly affect the antiphage activity. The monitoring of the intracellular phage infection process by DNA replication, gene expression, and electron microscopy as well as the study of phage mutants by genome mapping indicated that AbiTi is likely to act at a later stage of the phage lytic cycle.

- L35 ANSWER 7 OF 87 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN  
2002299347 EMBASE Functional domain of bovine milk lactoferrin which inhibits the adherence of Streptococcus mutans cells to a salivary film. Oho T.; Mitoma M.; Koga T. T. Oho, Department of Preventive Dentistry, Kyushu Univ. Fac. of Dental Science, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. oho@dent.kyushu-u.ac.jp. Infection and Immunity 70/9 (5279-5282) 2002.  
Refs: 21.  
ISSN: 0019-9567. CODEN: INFIBR. Pub. Country: United States. Language: English. Summary Language: English.
- AB The bovine lactoferrin molecule and relatively long lactoferrin fragments containing residues 473 to 538 strongly inhibited adherence of Streptococcus mutans to saliva-coated hydroxyapatite beads. Each cysteine residue in Lf411 (residues 473 to 538) was replaced by a serine residue, and the mutants Lf411-C481S and Lf411-C532S strongly inhibited S. mutans adherence. These results suggest that the functional domain of lactoferrin that binds to a salivary film lies in residues 473 to 538 and that the region might be concealed by disulfide bond formation between Cys481 and Cys532 in the Lf411 fragment.
- L35 ANSWER 8 OF 87 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
2003:93304 Document No.: PREV200300093304. Ligand based structural studies of the CB1 cannabinoid receptor. Picone, R. P.; Fournier, D. J.; Makriyannis, A. (1). (1) Center for Drug Discovery, University of Connecticut, 372 Fairfield Way, U2092, Storrs, CT, 06269-2092, USA: Makriyan@uconnvm.uconn.edu USA. Journal of Peptide Research, (December 2002, 2002) Vol. 60, No. 6, pp. 348-356. print. ISSN: 1397-002X. Language: English.
- AB The structural characterization of G-protein coupled receptors (GPCRs) is quite important as these proteins represent a vast number of therapeutic targets involved in drug discovery. However, solving the three-dimensional structure of GPCR has been a significant obstacle in structural biology. A variety of reasons, including their large molecular weight, intricate interhelical packing, as well as their membrane-associated topology, has hindered efforts aimed at their purification. In the absence of pure protein, available in the native conformation, classical methods of structural analysis such as X-ray crystallography and nuclear magnetic resonance spectroscopy cannot be utilized successfully. Alternative methods must therefore be explored to facilitate the structural features involved in drug-receptor interactions. The methods described herein detail the use of covalent probes, or affinity labels, capable of binding covalently to a target GPCR at its binding site(s). Our approach involves the incorporation of a number of reactive moieties in different regions of the ligand molecule each of which is expected to react with different amino acid residues. Information obtained from such work coupled with computer modeling and validated by the use of **site-directed mutagenesis** of GPCRs allows for three-dimensional mapping of the receptor binding site. It also sheds light on the different possible binding motifs for the various classes of agonists and antagonists and identifies amino acid residues involved with GPCR activation or inactivation.
- L35 ANSWER 9 OF 87 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
2002:326055 Document No.: PREV200200326055. Expression of bovine beta-lactoglobulin as a fusion protein in Escherichia coli: A tool for investigating how structure affects function. Ariyaratne, K. A. N. S.;

- Brown, Rosemary; Dasgupta, Arjit; de Jonge, Jorgen; Jameson, Geoffrey B.; Loo, Trevor S.; Weinberg, Cristina; Norris, Gillian E. (1). (1) Institute of Molecular Biosciences, Massey University, Palmerston North: g.norris@massey.ac.nz New Zealand. International Dairy Journal, (2002) Vol. 12, No. 4, pp. 311-318. <http://www.elsevier.com/locate/ldairyj>. print. ISSN: 0958-6946. Language: English.
- AB A synthetic gene for bovine beta-lactoglobulin-variant-A (beta-LgA), designed to incorporate a number of restriction sites to form suitable "cassettes" for future **site-directed mutagenesis** experiments, was constructed as three separate fragments, F1, F2 and F3. F1 was made using the shotgun approach while F2 and F3 were synthesised using PCR. The synthetic gene was cloned into the pTrxFus expression vector and recombinant protein was expressed in *Escherichia coli* (E. coli) as a fusion protein with thioredoxin (11.7 kDa). The fusion protein was purified using ion exchange chromatography (IEX) to give an yield of 100 mg of fusion protein per litre of culture. Recombinant bovine beta-LgA was cleaved from the fusion protein with enterokinase, then purified by IEX and size exclusion chromatography to give a final yield of 15 mg L<sup>-1</sup> of culture. Circular dichroism spectroscopy and mass spectrometry confirmed the moderate production of a correctly folded soluble full-length protein in an E. coli host.
- L35 ANSWER 10 OF 87 CAPLUS COPYRIGHT 2003 ACS on STN  
2003:101372 The key residues for conversion of electron acceptor specificity of xanthine oxidoreductase analysed by **site-directed mutagenesis**. Kuwabara, Yoshimitsu; Nishino, Tomoko; Okamoto, Ken; Araki, Tsutomu; Eger, Bryan T.; Pai, Emil F.; Nishino, Takeshi (Department of Biochemistry and Molecular Biology, Nippon Medical School, Tokyo, 113-8602, Japan). Flavins and Flavoproteins 2002, Proceedings of the International Symposium, 14th, Cambridge, United Kingdom, July 14-18, 2002, 275-279. Editor(s): Chapman, Stephen K.; Perham, Richard N.; Scrutton, Nigel S. Rudolf Weber, Agency for Scientific Publications: Berlin, Germany. ISBN: 3-00-010229-9 (English) 2002. CODEN: 69DPFY.
- AB A **site-directed mutagenesis** expt. was carried out and the mutant enzymes, R335A, W336A and R4276Q, were constructed to study the mechanism of transition of xanthine oxidoreductase. The redn. of sulfhydryl residues by treatment with DTT of mutant enzyme W336A led to only slight increase of the NAD-dependent activity and the O2-dependent activity remained still high. Partially purified mutant enzymes R335A and R427Q also resisted conversion from XO to xanthine dehydrogenase (XDH) by DTT treatment, but in a less pronounced manner than the W336A mutant. These results implied the involvement of Arg335, Trp336 and Arg427 in the XDH to XO transition mechanism as deduced from the analyses of the crystal structures of bovine **milk** XOR. The active site loop around the FAD is flexible in the mutant enzymes since they assume both XDH and XO configurations, even after DTT treatment. This would assign to Arg335, Trp336 and Arg427 an essential role in causing the active site to assume its unusual and tight XDH-type conformation.
- L35 ANSWER 11 OF 87 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
DUPLICATE 3  
2002:443027 Document No.: PREV200200443027. Proteolysis by lactoferrin: Insights into mechanism, and substrate specificity. St. Geme, J. W., III (1); Hendrixson, D. R.; Qiu, J.; Baker, E. N.; Plaut, A. G.. (1) Edward Mallinckrodt Department of Pediatrics and Department of Molecular Microbiology, Washington University School of Medicine, Saint Louis, MO, 63110 USA. Biochemistry and Cell Biology, (2002) Vol. 80, No. 1, pp. 156. print. Meeting Info.: 5th International Conference on Lactoferrin: Structure, Function and Applications Banff, Alberta, Canada May 04-09, 2001 ISSN: 0829-8211. Language: English.
- L35 ANSWER 12 OF 87 MEDLINE on STN  
2001687698 Document Number: 21576175. PubMed ID: 11567025. N-terminal processing is essential for release of epithin, a mouse type II membrane

serine protease. Cho E G; Kim M G; Kim C; Kim S R; Seong I S; Chung C; Schwartz R H; Park D. (School of Biological Sciences, Seoul National University, Seoul 151-742, Republic of Korea. ) JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Nov 30) 276 (48) 44581-9. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

- AB Epithin was originally identified as a mouse type II membrane serine protease. Its human orthologue membrane type-serine protease 1 (MT-SP1)/matriptase has been reported to be localized on the plasma membrane. In addition, soluble forms of matriptase were isolated from human breast milk and breast cancer cell-conditioned medium. In this paper, we report a processing mechanism that appears to be required for the release of epithin. CHO-K1 or COS7 cells transfected with single full-length epithin cDNA generated two different-sized proteins in cell lysates, 110 and 92 kDa. The 92-kDa epithin was found to be an N-terminally truncated form of the 110-kDa epithin, and it was the only form detected in the culture medium. The 92-kDa epithin was also found on the cell surface, where it was anchored by the N-terminal fragment. The results of in vivo cell labeling experiments indicate that the 110-kDa epithin is rapidly processed to the 92-kDa epithin. Using **site-directed mutagenesis** experiments, we identified Gly(149) of the GSIVIA sequence in epithin as required for the processing and release of the protein. These results suggest that N-terminal processing of epithin at Gly(149) is a necessary prerequisite step for release of the protein.

- L35 ANSWER 13 OF 87 MEDLINE on STN DUPLICATE 5  
2001443487 Document Number: 21381956. PubMed ID: 11488931. Improving solubility of catalytic domain of human beta-1,4-galactosyltransferase 1 through rationally designed amino acid replacements. Malissard M; Berger E G. (Institute of Physiology, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland. ) EUROPEAN JOURNAL OF BIOCHEMISTRY, (2001 Aug) 268 (15) 4352-8. Journal code: 0107600. ISSN: 0014-2956. Pub. country: Germany; Germany, Federal Republic of. Language: English.
- AB beta-1,4-galactosyltransferase 1 (beta4gal-T1, EC 2.4.1.38) transfers galactose from UDP-galactose to free N-acetyl-D-glucosamine or bound N-acetyl-D-glucosamine-R. Soluble beta4gal-T1, purified from human milk has been refractory to structural studies by X-ray or NMR. In a previous study (Malissard et al. 1996, Eur. J. Biochem. 239, 340-348) we produced in the yeast *Saccharomyces cerevisiae* an N-deglycosylated form of soluble beta4gal-T1 that was much more homogeneous than the human enzyme, as it displayed only two isoforms when analysed by IEF as compared to 13 isoforms for the native beta4gal-T1. The propensity of recombinant beta4gal-T1 to aggregate at concentrations > 1 mg.mL(-1) prevented structural and biophysical studies. In an attempt to produce a beta4gal-T1 form suitable for structural studies, we combined **site-directed mutagenesis** and heterologous expression in *Escherichia coli*. We produced a mutated form of the catalytic domain of beta4gal-T1 (sfbeta4gal-T1mut) in which seven mutations were introduced at nonconserved sites (A155E, N160K, M163T, A168T, T242N, N255D and A259T). Sfbeta4gal-T1mut was shown to be much more soluble than beta4gal-T1 expressed in *S. cerevisiae* (8.5 mg.mL(-1) vs. 1 mg.mL(-1)). Catalytic activity and kinetic parameters of sfbeta4gal-T1mut produced in *E. coli* were shown not to differ to any significant extent from those of the native enzyme.

- L35 ANSWER 14 OF 87 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
2001:251959 The Genuine Article (R) Number: 413QX. Mutations affecting the calcium-binding site of myeloperoxidase and lactoperoxidase. Shin K; Hayasawa H; Lonnerdal B (Reprint). Morinaga Milk Ind Co Ltd, Nutr Sci Lab, 5-1-83 Higashihara, Kanagawa 2288583, Japan (Reprint); Morinaga Milk Ind Co Ltd, Nutr Sci Lab, Kanagawa 2288583, Japan; Univ Calif Davis, Dept Nutr, Davis, CA 95616 USA. BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS (9 MAR 2001) Vol. 281, No. 4, pp. 1024-1029. Publisher: ACADEMIC PRESS INC. 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA. ISSN: 0006-291X. Pub. country: Japan; USA. Language: English.

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Both myeloperoxidase (MPO) and lactoperoxidase (LPO) contain high affinity bound calcium, which has been suggested to play a structural role. Asp-96 in MPO, a residue next to the histidine distal from the heme prosthetic group, has been assigned to the calcium-binding site of the enzyme by X-ray crystallography. Multiple sequence alignment of known animal peroxidases has revealed that the calcium-binding site is highly conserved. In this study, we replaced Asp-96 in MPO and the counterpart Asp-227 in LPO both with Ala by **site-directed mutagenesis**. The level of peroxidase activity in insect cells infected with recombinant baculoviruses and their culture supernatants was reduced to virtually zero as a result of these mutations. Immunoblotting revealed that these mutant peroxidases were expressed in the cells but not secreted as effectively as the wild-type enzymes. Our findings suggest that a functional calcium-binding site is essential for the biosynthesis of active animal peroxidases. (C) 2001 Academic Press.

L35 ANSWER 15 OF 87 MEDLINE on STN DUPLICATE 6  
2001537413 Document Number: 21226669. PubMed ID: 11328603. Replacements of amino acid residues at subsites and their effects on the catalytic properties of Rhizomucor pusillus pepsin, an aspartic proteinase from Rhizomucor pusillus. Aikawa J; Park Y N; Sugiyama M; Nishiyama M; Horinouchi S; Beppu T. (Department of Biotechnology, Graduate School of Agriculture and Life Sciences, and Biotechnology Research Center, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan..  
umanis@mail.ecc.u-tokyo.ac.jp) . JOURNAL OF BIOCHEMISTRY, (2001 May) 129 (5) 791-4. Journal code: 0376600. ISSN: 0021-924X. Pub. country: Japan. Language: English.

AB **Site-directed mutagenesis** was carried out to investigate the functional roles of amino acid residues of Rhizomucor pusillus pepsin (RMPP) in substrate-binding and catalysis. Mutations of two amino acid residues, E13 in the S3 subsite and N219 in the S3/S4 subsites, caused marked changes in kinetic parameters for two substrate peptides with different sequences. Further **site-directed mutagenesis** at E13 suggested that E13 plays a critical role in forming the correct hydrogen bond network around the active center. In the crystal structure of Rhizomucor miehei pepsin (RMPP), which is an aspartic proteinase produced by Rhizomucor miehei and shows 81% amino acid identity to RMPP, the Oepsilon atom of N219 forms a hydrogen bond with the N-H of isoleucine in pepstatin A, a statine-type inhibitor, at the P3 position, suggesting that the loss of the hydrogen bond causes an unfavorable arrangement of the P3 residue. Among the mutants constructed, the E13A mutant showed a 5-fold increase in the ratio of clotting versus proteolytic activity without significant loss of clotting activity. This mutant may present a promising candidate for a useful milk coagulant.

L35 ANSWER 16 OF 87 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
2001:439716 Document No.: PREV200100439716. Improved autoproducting efficiency of mutant subtilisins E with altered specificity by engineering of the pro-region. Takahashi, Masakazu (1); Hasuura, Yoshiyuki; Nakamori, Shigeru; Takagi, Hiroshi. (1) Department of Bioscience, Fukui Prefectural University, Fukui, 910-1195: mastak@fpu.ac.jp Japan. Journal of Biochemistry (Tokyo), (July, 2001) Vol. 130, No. 1, pp. 99-106. print. ISSN: 0021-924X. Language: English. Summary Language: English.

AB Modification of substrate specificity of an autoproducting enzyme is accompanied by a risk of significant failure of self-cleavage of the pro-region essential for activation. Therefore, to enhance processing, we engineered the pro-region of mutant subtilisins E of Bacillus subtilis with altered substrate specificity. A high-activity mutant subtilisin E with Ile31Leu replacement (I31L) as well as the wild-type enzyme show poor recognition of acid residues as the P1 substrate. To increase the P1 substrate preference for acid residues, Glu156Gln and Gly166Lys/Arg substitutions were introduced into the I31L gene based upon a report on subtilisin BPN' (Wells et al. (1987) Proc. Natl. Acad. Sci. USA 84,

1219-1223). The apparent P1 specificity of four mutants (E156Q/G166K, E156Q/G166R, G166K, and G166R) was extended to acid residues, but the halo-forming activity of *Escherichia coli* expressing the mutant genes on skim milk-containing plates was significantly decreased due to the lower autoproducting efficiency. A marked increase in active enzyme production occurred when Tyr(-1) in the pro-region of these mutants was then replaced by Asp or Glu. Five mutants with Glu(-2)Ala/Val/Gly or Tyr(-1)Cys/Ser substitution showing enhanced halo-forming activity were further isolated by PCR random mutagenesis in the pro-region of the E156Q/G166K mutant. These results indicated that introduction of an optimum arrangement at the cleavage site in the pro-region is an effective method for obtaining a higher yield of active enzymes.

L35 ANSWER 17 OF 87 CAPLUS COPYRIGHT 2003 ACS on STN

2000:441949 Document No. 133:70717 Chemical modification of enzymes with methanethiosulfonate reagents for adding multiple charges and altering specificity and/or activity. Davis, Benjamin G.; Jones, John Bryan; Bott, Richard R. (Genencor International, Inc., USA). PCT Int. Appl. WO 2000037658 A2 20000629, 93 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US30362 19991220. PRIORITY: US 1998-PV113130 19981221; US 1999-467536 19991220.

AB A method and reagent kit for altering specificity of enzymes by chem. modification using methanethiosulfonate reagents combined with genetic engineering based site-directed mutagenesis, is disclosed. One or more amino acid residues of enzymes are replaced by cysteine residues, where the cysteine residues are modified by replacing the thiol hydrogen in the cysteine residues with a substituent group providing a thiol side chain comprising a multiply charged moiety. Preferred enzymes are a serine hydrolase or a protease such as *Bacillus lentus* subtilisin, cellulase, amylase, lactase, or lipase, and the amino acid replacement occurs in the binding site. Preferred amino acids for replacement by cysteine are asparagine, leucine, methionine, or serine. In case of a trypsin-type serine protease, it also includes tyrosine, and glutamine, and for alpha/beta serine hydrolase such as *Candida antarctica* lipase, threonine, valine, isoleucine, and alanine. Neg. charges can be introduced by sulfonatoethyl thiol, 4-carboxybutyl thiol, 3,5-dicarboxybenzyl thiol, 3,3-dicarboxybutyl thiol, and 3,3,4-tricarboxybutyl thiol. Pos. charges can be introduced by aminoethyl thiol, 2-(trimethylammonium)ethyl thiol, 4,4-bis(aminomethyl)-3-oxo-hexyl thiol, and 2,2-bis(aminomethyl)-3-aminopropyl thiol. Those multiply charged moiety may be either a dendrimer or a polymer. A method of assaying for a preferred enzyme and detg. the catalytic efficiency of an enzyme by detg. the degree of stain removal from the material is also claimed. Use of detergent as component of enzyme-contg. compn. is claimed.

L35 ANSWER 18 OF 87 MEDLINE on STN

DUPLICATE 7

2001028723 Document Number: 20471908. PubMed ID: 11015192. Functional implications of disulfide bond, Cys206-Cys210, in recombinant prochymosin (chymosin). Chen H; Zhang G; Zhang Y; Dong Y; Yang K. (Institute of Microbiology, Chinese Academy of Sciences, Beijing 100080, China.) BIOCHEMISTRY, (2000 Oct 10) 39 (40) 12140-8. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB Prochymosin (chymosin) contains three disulfide bonds: Cys45-Cys50, Cys206-Cys210, and Cys250-Cys283. We have demonstrated that Cys250-Cys283 is indispensable for correct refolding of prochymosin, whereas Cys45-Cys50 is dispensable but has some contribution to the stability and substrate specificity of the enzyme. Here, we report the results about the

functions of Cys206-Cys210 by **site-directed mutagenesis** studies. In a glutathione redox system C206A/C210A mutant exhibited oxidative refolding kinetics and efficiency (approximately 40% reactivation) similar to those of the wild-type prochymosin, indicating that Cys206-Cys210 is also dispensable for refolding. However, C206S/C210S and single-site mutants (C210A, C210S, and C206A) showed only about 3 and 0-0.4% reactivation, respectively. This is quite different from the Cys45-Cys50 deficient mutants (C45A, C50A, C45A/C50A, C45D, C50S, C45D/C50S, C45A/C50S), which have comparable refolding efficiencies, implying that the substituents at position 206 and 210 play more important role in determining correct refolding than those at position 45 and 50. Urea-induced denaturation and fluorescence quenching studies indicated that the prochymosin mutants C206A/C210A and C206S/C210S were 2.1 and 4.8 kJ/mol less stable than prochymosin and some tryptophan residue in the mutated molecules was less exposed. However, the wild-type and mutant prochymosins shared similar far-UV CD and fluorescence emission spectra and similar specific potential activity, suggesting that the overall conformation was maintained after mutation. Activity assay and kinetic analysis revealed that mutation did not change the specific **milk-clotting** activity significantly but resulted in an increase in  $K(m)$  and  $k(cat)$  toward a hexapeptide substrate. On the basis of the above-mentioned perturbation of tryptophanyl microenvironment and the three-dimensional structure of chymosin, we proposed that deletion of Cys206-Cys210 may induce a propagated conformational change, resulting in a perturbation of the local conformation around active-site cleft and in turn, an alteration of the substrate specificity.

L35 ANSWER 19 OF 87 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STNDUPLICATE 8  
2000:233301 The Genuine Article (R) Number: 295EY. Biochemical characterization of PAI-2 and its mutants. Tian Y (Reprint); Shen J Q; Li P; Zhu Y S. SHANGHAI MED UNIV, BASIC MED SCH, MOL GENET LAB, SHANGHAI 200032, PEOPLES R CHINA. ACTA BIOCHIMICA ET BIOPHYSICA SINICA (MAR 2000) Vol. 32, No. 2, pp. 126-132. Publisher: SHANGHAI INST BIOCHEMISTRY, ACADEMIA SINICA. 320 YUE-YANG ROAD, SHANGHAI 20031, PEOPLES R CHINA. ISSN: 0582-9879. Pub. country: PEOPLES R CHINA. Language: Chinese.  
\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB To study and compare the biochemical characterization of PAI-2 and its mutants, PCR and **site-directed mutagenesis** methods were used to generate two PAI-2 mutants, PAI-2CD and PAI-2Q, respectively. The two mutant cDNAs were inserted into prokaryotic expression vector and expressed in a special strain of *E. coli*, JF1125. The expected PAI-2 mutant proteins were identified by SDS-PAGE, both covering about 14 % of total bacteria proteins. The antigenicity and activity inhibiting uPA of the two mutant proteins were verified to be identical with that of wild PAI-2 by using Western blot and **milk-agarose** plate assay and reverse **milk-agarose** zymograph. The harvested recombinant bacterial cells growing in 5 L fermenter were homogenized and purified by the protocols including ammonium sulfate precipitation, Sephadex G-75 gel filtration, Q-Sepharose ion-exchange chromatography and hydrophobic interaction chromatography. The purity of the purified PAI-2CD and PAI-2Q was up to 98 % and 90 %, the protein yield was 18.4 % and 22.1 %, the specific activity was 28 640 u/mg and 14 836 u/mg, respectively. The results indicate that the biochemical characterization of PAI-2 mutants was very similar to those of the wide-type PAI-2, except that PAI-2Q can not be catalyzed by tTG.

L35 ANSWER 20 OF 87 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN  
1999409237 EMBASE Mouse mammary tumor virus carrying a bacterial **spf** gene has wild-type pathogenicity and enables rapid isolation of proviral integration sites. Jiang Z.; Shackelford G.M.. G.M. Shackelford, Division of Hematology/Oncology, Childrens Hospital Los Angeles, 4650 Sunset Blvd., Los Angeles, CA 90027, United States. shacklef@hsc.usc.edu. Journal of Virology 73/12 (9810-9815) 1999.  
Refs: 34.  
ISSN: 0022-538X. CODEN: JOVIAM. Pub. Country: United States. Language:

English. Summary Language: English.

- AB Mouse mammary tumor virus (MMTV) has frequently been used as an insertional mutagen to identify provirally activated mammary proto-oncogenes. To expedite and facilitate the process of cloning MMTV insertion sites, we have introduced a bacterial supF suppressor tRNA gene into the long terminal repeat (LTR) of MMTV, thus allowing selection of clones containing it in lambda vectors bearing amber mutations. The presence of supF in the LTR should circumvent the screening process for proviral insertion sites, since only those lambda clones with supF-containing proviral-cellular junction fragments should be able to form plaques on a lawn of wild-type *Escherichia coli* (i.e., lacking supF). The resulting virus (MMTVsupF) induced mammary tumors at the expected rate in infected mice, deleted the appropriate T-cell population by virtue of its superantigen gene, and stably retained the supF gene after passage via the milk to female offspring. To test the selective function of the system, size-selected DNA containing two proviral-cellular junction fragments from an MMTV supF-induced mammary tumor was ligated into .lambda.gtwES..lambda.B, packaged, and plated on a supF-deficient bacterial host for selection of supF-containing clones. All plaques tested contained the desired cloned fragments, thus demonstrating the utility of this modified provirus for the rapid cloning of MMTV insertion sites.

L35 ANSWER 21 OF 87 CAPLUS COPYRIGHT 2003 ACS on STN

- 2001:581217 Document No. 135:354617 Crystal structure of xanthine oxidoreductase and EPR assignment of Fe/S centers. Enroth, Cristofor; Eger, Bryan T.; Pai, Emil F.; Okamoto, Ken; Iwasaki, Toshio; Nishino, Tomoko; Hori, Hiroyuki; Nishino, Takeshi (Department of Biochemistry, University of Toronto, Toronto, ON, M5S 1A8, Can.). Flavins and Flavoproteins 1999, Proceedings of the International Symposium, 13th, Konstanz, Germany, Aug. 29-Sept. 4, 1999, 783-786. Editor(s): Ghisla, Sandro. Rudolf Weber, Agency for Scientific Publications: Berlin, Germany. (English) 1999. CODEN: 69BQDP.

- AB The crystal structure of bovine milk xanthine oxidoreductase (XOR) was solved and was found to be a butterfly-shaped homodimer of overall dimensions 155 .times. 90 .times. 70 .ANG.; the model of the dehydrogenase form was refined to 2.1 .ANG. resolu. The Cys ligand residues to the individual [2Fe-2S] clusters of XOR were assigned by a combination of site-directed mutagenesis and ESR spectroscopy; Cys-115 was found to serve as one of the Cys ligands to the Fe/S I center and Cys-43 was one of the Cys ligands to the Fe/S II center. The results of the crystallog. and ESR studies indicated that the intramol. electron transfer processes occur in the sequence, molybdopterine .fwdarw. Fe/S I center .fwdarw. Fe/S II center .fwdarw. FAD.

L35 ANSWER 22 OF 87 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN

- 2000:23068 The Genuine Article (R) Number: 268XF. Zona pellucida glycoprotein mZP3 produced in milk of transgenic mice is active as a sperm receptor, but can be lethal to newborns. Litscher E S; Liu C Y; Echelard Y; Wassarman P M (Reprint). MT SINAI SCH MED, DEPT BIOCHEM & MOL BIOL, 1 GUSTAVE L LEVY PL, NEW YORK, NY 10029 (Reprint); MT SINAI SCH MED, DEPT BIOCHEM & MOL BIOL, NEW YORK, NY 10029. TRANSGENIC RESEARCH (OCT 1999) Vol. 8, No. 5, pp. 361-369. Publisher: KLUWER ACADEMIC PUBL. SPUIBOULEVARD 50, PO BOX 17, 3300 AA DORDRECHT, NETHERLANDS. ISSN: 0962-8819. Pub. country: USA. Language: English.

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

- AB Mouse egg zona pellucida glycoprotein mZP3 (similar to 83 kDa M-r) serves as a species-specific sperm receptor and acrosome reaction-inducer during fertilization in mice. These biological activities are dependent on certain mZP3 serine/threonine- (O-) linked oligosaccharides present at the combining-site for sperm. In an attempt to produce large amounts of biologically active mZP3, we generated several transgenic mouse lines carrying the full-length mZP3 gene fused to the beta-casein gene promoter and transcription termination sequence. We found that different transgenic mouse lines have different amounts of recombinant mZP3 (similar to 63 kDa M-r) in milk of lactating females, from similar to 0.3 to 3.5 mu



g/mu l of milk. In all cases, purified milk-mZP3 is active as a sperm receptor and acrosome reaction-inducer in vitro. Unexpectedly, we also found that development of litters from these transgenic mice is related to the amount of mZP3 in the mother's milk. In the most extreme case, litters from the highest expressers fail to live beyond about day-7 post partum unless placed immediately after birth with surrogate wild-type mothers. Litters from lower expressers initially display a complex phenotype that includes effects on hair and body growth, but some of the mice survive and, in time, are restored to a wild-type phenotype. These results demonstrate that relatively large amounts of biologically active mZP3 can be produced in transgenic mouse milk for structural and other studies, but that the presence of mZP3 in milk has dramatic developmental effects on litters, ranging from retarded hair and body growth to death of newborn pups.

L35 ANSWER 23 OF 87 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN 1999:300867 Document No.: PREV199900300867. Construction and characterization of a chimeric fusion protein consisting of an anti-idiotypic antibody mimicking a breast cancer-associated antigen and the cytokine GM-CSF. Tripathi, Pulak K.; Qin, Hongxing; Bhattacharya-Chatterjee, Malaya; Ceriani, Roberto L.; Foon, Kenneth A.; Chatterjee, Sunil K. (1). (1) Markey Cancer Center, University of Kentucky, 204 Combs Research Building, Lexington, KY, 40536-0096 USA. Hybridoma, (April, 1999) Vol. 18, No. 2, pp. 193-202. ISSN: 0272-457X. Language: English. Summary Language: English.

AB Anti-idiotypic antibody, 11D10 mimics biologically and antigenically a distinct and specific epitope of the high molecular weight human milk fat globule (HMFG), a cancer-associated antigen present in over 90% of breast tumor samples. To augment the immunogenicity of 11D10 without the aid of a carrier protein or adjuvant, we made a chimeric 11D10-GM-CSF fusion protein for use as a vaccine. An expression plasmid for 11D10 was made by ligation of the DNA sequences of the 11D10 light-chain variable region upstream of the human kappa constant region. The heavy-chain plasmid carrying GM-CSF was made by ligation of the heavy-chain variable region sequences upstream of the human gamma1 constant region CH1 fused to the DNA fragment encoding the mature GM-CSF peptide 3' to the CH3 exon. NS1 plasmacytoma cells were transfected with the light and heavy-chain vectors by electroporation. Fusion protein secreted in the culture medium was purified and was characterized by gel electrophoresis as well as by determination of the biological activity of the fused GM-CSF. In nonreducing SDS-polyacrylamide gels, a single band approx 200 Kd reacted with anti-human kappa, anti-human lambda and anti-GM-CSF antibodies. In reducing polyacrylamide gels, a approx 74 kd protein reacted with anti-human lambda and anti-GM-CSF antibodies. The fusion protein induced proliferation of GM-CSF dependent NFS-60 cells. These results suggest that the protein is a chimeric anti-idiotypic antibody consisting of 11D10 variable domains, human kappa and lambda constant domains and that the GM-CSF moiety fused to the constant region lambda is biologically active.

L35 ANSWER 24 OF 87 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN 1999:146839 EMBASE Human growth hormone site 2 lactogenic activity requires a distant tyrosine164. Duda K.M.; Brooks C.L. C.L. Brooks, Ohio State Biochemistry Program, Ohio State University, 1925 Coffey Road, Columbus, OH 43210, United States. brooks.8@osu.edu. FEBS Letters 449/2-3 (120-124) 1999.

Refs: 22.  
ISSN: 0014-5793. CODEN: FEBLAL.  
Publisher Ident.: S 0014-5793(99)00416-0. Pub. Country: Netherlands.  
Language: English. Summary Language: English.

AB Comparison of crystallographic structures of human growth hormone, either bound to the prolactin receptor or free of receptors, reveals that human growth hormone binding to the prolactin receptor at site 1 is associated with a structural change in human growth hormone that influences the

organization of residues which constitute site 2. We have identified Tyr164 as a residue that is critical for the propagation of this structural rearrangement. Tyr164 is a structural epitope for site 1 and is distal to site 2. Mutation of Tyr164 to glutamic acid (Y164E) does not affect the somatotrophic activity, absorption or fluorescence spectra or binding to the human prolactin receptor when compared to wild-type human growth hormone, indicating the subtle effects of the mutation. Lactogenic assays using extended concentrations of Y164E human growth hormone produce dose-response curves that are characterized by a right-shifted agonist phase and an unchanged antagonist phase when compared to wild-type human growth hormone. These results indicate that Tyr164 is required for the lactogenic activity of human growth hormone and that mutation to glutamic acid disrupts the lactogenic function of site 2. Copyright (C) 1999 Federation of European Biochemical Societies.

L35 ANSWER 25 OF 87 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
1999:650236 The Genuine Article (R) Number: 227WQ. Fine tuning the N-terminus of a calcium binding protein: alpha-lactalbumin. Vepintsev D B; Narayan M; Permyakov S E; Uversky V N; Brooks C L; Cherskaya A M; Permyakov E A; Berliner L J (Reprint). OHIO STATE UNIV, DEPT CHEM, 100 W 18TH AVE, COLUMBUS, OH 43210 (Reprint); OHIO STATE UNIV, DEPT CHEM, COLUMBUS, OH 43210; RUSSIAN ACAD SCI, INST BIOL INSTRUMENTAT, PUSHCHINO 142292, RUSSIA; OHIO STATE UNIV, DEPT VET BIOSCI, COLUMBUS, OH 43210. PROTEINS-STRUCTURE FUNCTION AND GENETICS (1 OCT 1999) Vol. 37, No. 1, pp. 65-72. Publisher: WILEY-LISS. DIV JOHN WILEY & SONS INC, 605 THIRD AVE, NEW YORK, NY 10158-0012. ISSN: 0887-3585. Pub. country: USA; RUSSIA. Language: English.  
\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The effects of amino acid substitutions in the N-terminus of bovine recombinant  $\alpha$ -lactalbumin (including enzymatic removal of the N-terminal methionine and deletion of Glu-1) were studied by intrinsic fluorescence, circular dichroism (CD), and differential scanning microcalorimetry (DSC). Wild-type recombinant alpha-lactalbumin has a lower thermostability and calcium affinity compared to the native protein, while the properties of wild-type protein with the N-terminal methionine enzymatically removed are similar to the native protein. Taken together, the fluorescence, CD, and DSC results show that recombinant wild type alpha-lactalbumin in the absence of calcium ion is in a type of molten globule state. The delta-E1 mutant, where the Glu(1) residue of the native sequence is genetically removed, leaving an N-terminal methionine in its place, shows almost one order of magnitude higher affinity for calcium and higher thermostability (both in the absence and presence of calcium) than the native protein isolated from milk. It was concluded that the N-terminus of the protein dramatically affects both stability and function as manifested in calcium affinity. (C) 1999 Wiley-Liss, Inc.

L35 ANSWER 26 OF 87 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
1999:429351 The Genuine Article (R) Number: 200NH. Introduction of a proximal Stat5 site in the murine alpha-lactalbumin promoter induces prolactin dependency in vitro and improves expression frequency in vivo. Soulier S; Lepourry L; Stinnakre M G; Langley B; Lhuillier P J; Paly J; Djiane J; Mercier J C; Villette J L (Reprint). INRA, LAB GENET BIOCHIM & CYTOGENET, F-78352 JOUY EN JOSAS, FRANCE (Reprint); INRA, LAB GENET BIOCHIM & CYTOGENET, F-78352 JOUY EN JOSAS, FRANCE; AGRIS, RUAKURA RES CTR, HAMILTON, NEW ZEALAND; INRA, BIOL CELLULAIRE & MOL LAB, F-78352 JOUY EN JOSAS, FRANCE. TRANSGENIC RESEARCH (FEB 1999) Vol. 8, No. 1, pp. 23-31. Publisher: KLUWER ACADEMIC PUBL. SPUISBOULEVARD 50, PO BOX 17, 3300 AA DORDRECHT, NETHERLANDS. ISSN: 0962-8819. Pub. country: FRANCE; NEW ZEALAND. Language: English.  
\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB In order to establish a possible correlation between in vitro prolactin induction and the transcriptional activity of mammary gene promoters in transgenic mice, a functional Stat5-binding site was created by means of site-directed mutagenesis at position -70 on a 560 bp murine cc-lactalbumin promoter linked to a CAT reporter gene. Surprisingly, the wild-type promoter was constitutively active in vitro

and could not be induced by prolactin. Introducing the proximal Stat5 site abolished this constitutive activity and resulted in prolactin dependence in both CHO-K1- and HC11-transfected cells. In transgenic mice, both the frequency of lines expressing the transgene and the prevalence of mid to late pregnancy expression were increased.

L35 ANSWER 27 OF 87 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN  
1998:308553 EMBASE The diversity of lipases from psychrotrophic strains of pseudomonas: A novel lipase from a highly lipolytic strain of Pseudomonas fluorescens. Dieckelmann M.; Johnson L.A.; Beacham I.R. Dr. I.R. Beacham, School of Health Science, Griffith University, Gold Coast Campus, PMB 50, Gold Coast Mail Centre, QLD 4217, Australia. Journal of Applied Microbiology 85/3 (527-536) 1998.

Refs: 38.

ISSN: 1364-5072. CODEN: JAMIFK. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB Strains of Pseudomonas fluorescens and Ps. fragi are the predominant psychrotrophs found in raw milk and may cause spoilage due to the secretion of hydrolytic enzymes such as lipase and protease. The diversity of lipases has been examined in Pseudomonas isolates from raw milk which represent different taxonomic groups (phenons). Significant diversity was found using both DNA hybridization and immunoblotting techniques, which has implications for the development of a diagnostic test. The lipase-encoding gene (lipA) was cloned from one strain, C9, of Ps. fluorescens biovar V. In contrast to previously reported lipase sequences from Ps. fluorescens, the gene encodes a lipase of M(r) 33 kDa. Alignment of all known Pseudomonas and Burkholderia lipase amino acid sequences indicates the existence of two major groups, one of M(r) approximately 30 kDa comprising sequences from Ps. fragi, Ps. aeruginosa, Ps. fluorescens C9 and Burkholderia, and one of approximately 50 kDa comprising Ps. fluorescens lipases. The lipase from C9 does not contain a signal peptide and is presumed to be secreted via a signal peptide-independent pathway. The lipA gene of strain C9 was disrupted by insertional mutagenesis. The mutant retained its lipolytic phenotype, strongly suggesting the presence of a second lipase in this strain.

L35 ANSWER 28 OF 87 CAPLUS COPYRIGHT 2003 ACS on STN  
1998:700165 Document No. 129:329746 Structure function relationships in milk-clotting enzymes: pepsin-a model. Yada, R. Y.; Tanaka, T. (Department of Food Science, University of Guelph, Guelph, ON, N1G 2W1, Can.). ACS Symposium Series, 708 (Functional Properties of Proteins and Lipids), 122-144 (English) 1998. CODEN: ACSMC8. ISSN: 0097-6156. Publisher: American Chemical Society.

AB Numerous studies examg. functionality on a mechanistic level of food-related proteins have recently been undertaken using genetic engineering techniques. Research in our lab. has concd. on the genetic engineering of pepsin, a milk-clotting enzyme which belongs to aspartic proteinases, in order to det. the role of specific amino acids/regions of pepsin, and its zymogen, pepsinogen, on structure and catalytic activity. Results from these studies indicated that site-directed mutagenesis of regions both adjacent (e.g., flap loop region) and remote (e.g., prosegment of the zymogen, surface of the enzyme) to the active site were crit. to catalytic parameters and were reflected in structural changes as detd. by CD and mol. modeling. Information gleaned from such studies may allow us to redesign enzymes, as well as other food-related proteins, for a particular function and/or environment in a predictable manner.

L35 ANSWER 29 OF 87 CAPLUS COPYRIGHT 2003 ACS on STN  
1998:225374 Document No. 129:2135 Effect of replacement of the conserved Tyr75 on the catalytic properties of porcine pepsin A. Tanaka, Takuji; Teo, Karen S. L.; Lamb, Kyra M.; Harris, Linda J.; Yada, Rickey Y. (Department of Food Science, University of Guelph, Guelph, ON, N1G 2W1, Can.). Protein and Peptide Letters, 5(1), 19-26 (English) 1998. CODEN:

PPLEN. ISSN: 0929-8665. Publisher: Bentham Science Publishers.  
AB Replacement of a conserved Tyr 75 on the flap loop of pepsin resulted in a change of the catalytic rate. PH dependency and CD analyses indicated, however, that the effect was not global. Mol. modeling calcn. suggested that the replacements allowed the loop to assume another position thereby changing the catalytic efficiency.

L35 ANSWER 30 OF 87 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
97:624216 The Genuine Article (R) Number: XR221. Promoter-dependent synergy between glucocorticoid receptor and Stat5 in the activation of beta-casein gene transcription. Lechner J; Welte T; Tomasi J K; Bruno P; Cairns C; Gustafsson J A; Doppler W (Reprint). INNSBRUCK UNIV, INST MED CHEM & BIOCHEM, FRITZ PREGL STR 3, A-6020 INNSBRUCK, AUSTRIA (Reprint); INNSBRUCK UNIV, INST MED CHEM & BIOCHEM, A-6020 INNSBRUCK, AUSTRIA; HUDDINGE UNIV HOSP, KAROLINSKA INST, DEPT MED NUTR, NOVUM, S-14186 HUDDINGE, SWEDEN. JOURNAL OF BIOLOGICAL CHEMISTRY (15 AUG 1997) Vol. 272, No. 33, pp. 20954-20960. Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC. 9650 ROCKVILLE PIKE, BETHESDA, MD 20814. ISSN: 0021-9258. Pub. country: AUSTRIA; SWEDEN. Language: English.

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*  
AB Steroid hormone receptors and Stat factors comprise two distinct families of inducible transcription factors. Activation of a member of each family, namely the glucocorticoid receptor by glucocorticoids and Stat5 by prolactin, is required for the efficient induction of the expression of milk protein genes in the mammary epithelium. We have studied the mode of interaction between Stat5 and the glucocorticoid receptor in the activation of beta-casein gene transcription. The functional role of potential half-palindromic glucocorticoid receptor-binding sites mapped previously in the promoter region was investigated. beta-Casein gene promoter chloramphenicol acetyltransferase constructs containing mutations and deletions in these sites were tested for their responsiveness to the synergistic effect of prolactin and dexamethasone employing COS-7 cells or HC11 mammary epithelial cells. Synergism depended on promoter regions containing intact binding sites for the glucocorticoid receptor and Stat5. The carboxyl terminal transactivation domains of Stat5a and Stat5b were not required for this synergism. Our results suggest that in lactogenic hormone response elements glucocorticoid receptor molecules bound to nonclassical half-palindromic sites gain competence as transcriptional activators by the interaction with Stat5 molecules binding to vicinal sites.

L35 ANSWER 31 OF 87 MEDLINE on STN DUPLICATE 9  
97438507 Document Number: 97438507. PubMed ID: 9292995. Biochemical and molecular characterization of PepR, a dipeptidase, from *Lactobacillus helveticus* CNR232. Shao W; Yuksel G U; Dudley E G; Parkin K L; Steele J L. (Department of Food Science, University of Wisconsin-Madison 53706, USA.) APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (1997 Sep) 63 (9) 3438-43. Journal code: 7605801. ISSN: 0099-2240. Pub. country: United States. Language: English.

AB A dipeptidase with prolinase activity from *Lactobacillus helveticus* CNR232, which was designated PepR, was purified to gel electrophoretic homogeneity and characterized. The NH2-terminal amino acid sequence of the purified protein had 96% identity to the deduced NH2-terminal amino acid sequence of the pepR gene, which was previously designated pepPN, from *L. helveticus* CNR232. The purified enzyme hydrolyzed Pro-Met, Thr-Leu, and Ser-Phe as well as dipeptides containing neutral, nonpolar amino acid residues at the amino terminus. Purified PepR was determined to have a molecular mass of 125 kDa with subunits of 33 kDa. The isoelectric point of the enzyme was determined to be 4.5. The optimal reaction conditions, as determined with Pro-Leu as substrate, were pH 6.0 to 6.5 and 45 to 50 degrees C. The purified PepR had a Km of 4.9 to 5.2 mM and a Vmax of 260 to 270  $\mu$ mol of protein per min/mg at pH 6.5 and 37 degrees C. The activity of purified PepR was inhibited by Zn2+ but not by other cations or cysteine, serine, aspartic, or metal-containing protease inhibitors or reducing agents. Results obtained by site-

**directed mutagenesis** indicated that **PepR** is a serine-dependent protease. Gene replacement was employed to construct a **PepR**-deficient derivative of **CNR32**. This mutant did not differ from the wild-type strain in its ability to acidify **milk**. However, the **PepR**-deficient construct was determined to have reduced dipeptidase activity compared to the wild-type strain with all dipeptide substrates examined.

L35 ANSWER 32 OF 87 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
97:577239 The Genuine Article (R) Number: XM613. An angiogenic protein from bovine serum and **milk** - Purification and primary structure of angiogenin-2. Strydom D J; Bond M D; Vallee B L (Reprint). HARVARD UNIV, SCH MED, CTR BIOCHEM & BIOPHYS SCI & MED, 250 LONGWOOD AVE, BOSTON, MA 02115 (Reprint); HARVARD UNIV, SCH MED, CTR BIOCHEM & BIOPHYS SCI & MED, BOSTON, MA 02115; HARVARD UNIV, SCH MED, DEPT PATHOL, BOSTON, MA 02115. EUROPEAN JOURNAL OF BIOCHEMISTRY (15 JUL 1997) Vol. 247, No. 2, pp. 535-544. Publisher: SPRINGER VERLAG. 175 FIFTH AVE, NEW YORK, NY 10010. ISSN: 0014-2956. Pub. country: USA. Language: English.  
\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Bovine serum and **milk** contain a basic angiogenic protein that binds tightly to placental ribonuclease inhibitor. It was purified from both sources by ion-exchange and reversed-phase chromatographies. Its amino acid sequence revealed that it is a member of the ribonuclease superfamily. It contains 123 amino acids in a single polypeptide chain, is cross-linked by three disulfide bonds, is glycosylated at Asn33, and is 57% identical to bovine angiogenin. The amino-terminal and carboxyl-terminal residues are pyroglutamic acid and proline, respectively. The protein has ribonucleolytic activity that is similar to, but somewhat lower than, that of bovine angiogenin, i.e. very low relative to RNase. It is angiogenically potent on chicken chorioallantoic membrane, but less so than angiogenin. The sequence and activities demonstrate that this protein is a second, distinct, member of the angiogenin sub-family of pancreatic ribonucleases, and is referred to as angiogenin-2.

L35 ANSWER 33 OF 87 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN  
1998181024 EMBASE Pyroglutamic acid and iron regulate the expression of the **pcp** gene in *Pseudomonas fluorescens* MFO. Le Saux O.; Robert-Baudouy J. O. Le Saux, Pacific Biomedical Research Center, Laboratory of Matrix Pathobiology, 1993 East-West Road, Honolulu, HI 96822, United States. olivier@pbrc.hawaii.edu. FEMS Microbiology Letters 155/2 (209-215) 15 Oct 1997.  
Refs: 24.  
ISSN: 0378-1097. CODEN: FMLED7.  
Publisher Ident.: S 0378-1097(97)00389-3. Pub. Country: Netherlands.  
Language: English. Summary Language: English.

AB Pyrrolidone carboxyl peptidase (**Pcp**) is an aminopeptidase (EC 3.4.11.8) able to specifically remove the L-pyroglutamyl residue from the amino-terminus of polypeptides. Since nothing was known concerning the regulation and function of **Pcps**, a mutant of a **milk**-isolated strain lacking **Pcp** activity (*Pseudomonas fluorescens* MB1), was constructed by homologous recombination using a transcriptional fusion between **pcp** and a reporter gene (**uidA**). The wild-type and mutant strains were grown in synthetic media and in **milk** to investigate the environmental effects on **pcp** transcription. The expression of **pcp** and of the transcriptional fusion **pcp::uidA** was not sensitive to environmental conditions like temperature, osmolarity or nitrogen and phosphate starvation but was induced by the product of the enzymatic activity, pyroglutamic acid (**pGlu**). The expression of the native gene and the fusion in inducing conditions was also controlled by the iron concentration. The identification in the **pcp** promoter sequence of putative ferric uptake regulator (**Fur**) binding sites suggests a transcriptional regulation in a **Fur**-dependent fashion. Two other putative regulatory stretches, corresponding to inverted repeated sequences with perfect and imperfect symmetry, were also identified. **pGlu** and iron are therefore at least two of the transcriptional effectors of **pcp** expression.

L35 ANSWER 34 OF 87 MEDLINE on STN DUPLICATE 10  
 97223808 Document Number: 97223808. PubMed ID: 9056485. Characterization of human lactoferrin produced in the baculovirus expression system. Salmon V; Legrand D; Georges B; Slomianny M C; Coddeville B; Spik G. (Laboratoire de Chimie Biologique, Centre National de la Recherche Scientifique, Université des Sciences et Technologies de Lille, Villeneuve, France. ) PROTEIN EXPRESSION AND PURIFICATION, (1997 Mar) 9 (2) 203-10. Journal code: 9101496. ISSN: 1046-5928. Pub. country: United States. Language: English.

AB Lactoferrin, an iron-binding 80-kDa glycoprotein, is a major component of human milk whose structure is now well defined. The binding site of lactoferrin to the membrane receptor of lymphocyte has been located in the region 4-52, but the amino acids directly involved in the interaction have not been identified yet. To gain further insights into the structure-function relationships of the lactoferrin binding site, we first expressed the cDNA encoding human lactoferrin in the lepidoptera *Spodoptera frugiperda* cells (Sf9) using a recombinant baculovirus. The selected transformant secreted and N-glycosylated protein of 78 kDa which was immunoprecipitated by specific anti-lactoferrin antibodies. To confirm the structure and the function of the recombinant lactoferrin, the protein was purified by ion-exchange chromatography and its physical, biochemical, and biological properties were compared with those of the native protein. In particular, the N-terminal amino acid sequence and the iron-binding stability as a function of pH, of both proteins, were identical. The main difference concerns the glycosylation which leads to glycans of lower molecular masses as detected by the electrophoretic mobility of lactoferrin after N-glycosidase F treatment and matrix-assisted laser desorption ionization/time-of-flight mass spectrometry. Despite the different glycosylation features, the recombinant lactoferrin retained the binding property to the Jurkat human lymphoblastic T-cell line of the native lactoferrin. On the basis of these analyses, production of protein mutants generated by site-directed mutagenesis is now in process.

L35 ANSWER 35 OF 87 MEDLINE on STN DUPLICATE 11  
 97211203 Document Number: 97211203. PubMed ID: 9058201. Site-directed mutagenesis of conserved Trp39 in Rhizomucor pusillus pepsin: possible role of Trp39 in maintaining Tyr75 in the correct orientation for maximizing catalytic activity. Park Y N; Aikawa J; Nishiyama M; Horinouchi S; Beppu T. (Department of Biotechnology, University of Tokyo. ) JOURNAL OF BIOCHEMISTRY, (1997 Jan) 121 (1) 118-21. Journal code: 0376600. ISSN: 0021-924X. Pub. country: Japan. Language: English.

AB Replacement of Trp39 of Rhizomucor pusillus pepsin (RMPP) by Asn or Cys resulted in a marked decrease in the milk-clotting and proteolytic activities. Kinetic analysis with chromogenic synthetic oligopeptides as substrates revealed that the mutations caused marked changes in the *k*<sub>cat</sub> value, but only slight changes in the *K*<sub>m</sub> value. Similar enzymatic properties were observed in mutants of Tyr75, which was shown to have a role in enhancing the catalytic activity. Both Tyr75Asn and Trp39Asn mutants rapidly lost the activity at high temperatures due to autocatalytic digestion at two sites. The structures of several aspartic proteinases including RMPP, as revealed by X-ray crystallographic studies, showed that Trp39 occupies a position close to Tyr75 and the N delta atom of Trp39 within hydrogen-bonding distance of the hydroxyl side chain of Tyr75. These observations suggest that Trp39 plays a role in maintaining Tyr75 in the correct orientation in aspartic proteinases, including RMPP.

L35 ANSWER 36 OF 87 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
 96:416489 The Genuine Article (R) Number: UN474. COOPERATIVE INTERACTIONS BETWEEN THE AMINO-TERMINAL AND CARBOXYL-TERMINAL LOBES CONTRIBUTE TO THE UNIQUE IRON-BINDING STABILITY OF LACTOFERRIN. WARD P P; ZHOU X; CONNEELY O M (Reprint). BAYLOR COLL MED, DEPT CELL BIOL, 1 BAYLOR PLAZA, HOUSTON, TX, 77030 (Reprint); BAYLOR COLL MED, DEPT CELL BIOL, HOUSTON, TX, 77030.

AB Lactoferrin is a member of the transferrin family of iron-binding proteins. Several functions have been ascribed to lactoferrin, including regulation of iron homeostasis, antibacterial properties, and regulation of myelopoiesis. However, the structural features of lactoferrin that are required for most of these functions are unknown.

Previously, we reported the development of an efficient fungal expression system to produce recombinant human lactoferrin. The availability of this production system demonstrated the feasibility of producing mutant lactoferrins to address the structure/function relationship of the protein. In the present study, we used a **site-directed mutagenesis** approach to address the contribution of the bilobal structure of lactoferrin to its unique iron-binding stability. Like transferrin, lactoferrin consists of two repeated iron-binding lobes that bind one iron atom each. However, unlike transferrin, lactoferrin retains iron over a broad pH range, a key property that contributes to the unique iron-binding functions of the protein. Using mutants that selectively ablate the iron-binding function in either lobe, we demonstrate differential iron-binding stability of the amino- and carboxyl-terminal iron-binding lobes of lactoferrin. Further, we show that the unique iron-binding stability of the protein is imparted primarily by the carboxyl-terminal domain which functions cooperatively to stabilize iron-binding to the amino-terminal domain of lactoferrin.

L35 ANSWER 37 OF 87 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
DUPLICATE 12

1996:186466 Document No.: PREV199698742595. Genetic modification of bovine beta-casein and its expression in the **milk** of transgenic mice. Choi, Byung-Kwon; Bleck, Gregory T.; Wheeler, Matthew B.; Jimenez-Flores, Rafael (1). (1) Dep. Dairy Sci., California Polytechnic State Univ., San Luis Obispo, CA 93407 USA. Journal of Agricultural and Food Chemistry, (1996) Vol. 44, No. 3, pp. 953-960. ISSN: 0021-8561. Language: English.

AB Genomic vectors containing mutant bovine beta-casein with putative glycosylation sites were constructed to study the functional properties of glycosylated beta-casein and its possible effects in **milk**. The mutation was performed by PCR-based **site-directed mutagenesis**. The tripeptide sequence, Asn-X-Ser, was generated between Asn-68 and Asn-73 in mature beta-casein. The resulting beta-casein mutants were designated pCJB68 and pCJB6873. pCJB68 carries a substitution of Ser-70 for Leu-70 (Asn-68-Ser-69-Ser-70-Pro-71), and pCJB6873 carries a substitution of Ser-70-Ser-71 for Leu-70-Pro-71 (Asn-68-Ser-69-Ser-70-Ser-71). The two mutated genomic constructs were placed under control of the bovine alpha-lactalbumin promoter, and lines of mice expressing the pCJB68 and pCJB6873 have been established. The **milk** from transgenic mice contained bovine beta-casein at levels up to 2-3 mg/mL. N-Linked glycosylation of bovine beta-casein in the pCJB6873 line was confirmed by peptide-N-glycosidase F treatment, but glycosylation of bovine beta-casein did not occur in pCJB68 mice. In addition, mouse casein micelles containing glycosylated bovine beta-casein showed the largest median diameter and rough outer surface, compared to normal mouse casein micelles and micelles from transgenic **milk** containing bovine beta-casein.

L35 ANSWER 38 OF 87 MEDLINE on STN Duplicates 13  
96190739 Document Number: 96190739. PubMed ID: 8611177. Expression and secretion of recombinant ovine beta-lactoglobulin in *Saccharomyces cerevisiae* and *Kluyveromyces lactis*. Rocha T L; Paterson G; Crimmins K; Boyd A; Sawyer L; Fothergill-Gilmore L A. (Edinburgh Centre for Molecular Recognition, University of Edinburgh, Scotland, U.K.) BIOCHEMICAL JOURNAL, (1996 Feb 1) 313 ( Pt 3) 927-32. Journal code: 2984726R. ISSN: 0264-6021. Pub. country: ENGLAND: United Kingdom. Language: English.

AB High expression and secretion of recombinant ovine beta-lactoglobulin has been achieved in the yeast *Kluyveromyces lactis*. The yield of beta-lactoglobulin is 40-50 mg per litre of culture supernatant and

accounts for approx. 72% of the total secreted protein. Constitutive expression is under the control of the *Saccharomyces cerevisiae* phosphoglycerate kinase promoter from an intronless version of the beta-lactoglobulin gene. Secretion is specified by the ovine protein's own signal sequence. This system, coupled to an efficient and novel recovery protocol, allows 30 mg of pure protein to be isolated from a typical 1 litre culture. The protein is virtually indistinguishable from beta-lactoglobulin conventionally purified from sheep milk by its behaviour in native PAGE and SDS/PAGE, reactivity to antibodies, CD, fluorescence spectroscopy and N-terminal sequencing. Attempts to achieve a similar expression and secretion system in the yeast *S. cerevisiae* met with only limited success, although it was found that heat-shock treatment modestly increased the yield up to approx. 3-4 mg per litre of culture supernatant. **Site-directed mutagenesis** showed that secretion in *S. cerevisiae* depended upon correct formation of the two disulphide bonds present in beta-lactoglobulin.

- L35 ANSWER 39 OF 87 MEDLINE on STN DUPLICATE 14  
 97084794 Document Number: 97084794. PubMed ID: 8931128. Protein engineering loops in aspartic proteinases: **site-directed mutagenesis**, biochemical characterization and X-ray analysis of chymosin with a replaced loop from rhizopuspepsin. Nugent P G; Albert A; Orprayoon P; Wilsher J; Pitts J E; Blundell T L; Dhanaraj V. (Department of Crystallography, Birkbeck College, University of London, UK. ) PROTEIN ENGINEERING, (1996 Oct) 9 (10) 885-93. Journal code: 8801484. ISSN: 0269-2139. Pub. country: ENGLAND: United Kingdom. Language: English.
- AB The loop exchange mutant chymosin 155-164 rhizopuspepsin was expressed in *Trichoderma reesei* and exported into the medium to yield a correctly folded and active product. The biochemical characterization and crystal structure determination at 2.5 Å resolution confirm that the mutant enzyme adopts a native fold. However, the conformation of the mutated loop is unlike that in native rhizopuspepsin and involves the chelation of a water molecule in the loop. Kinetic analysis using two synthetic peptide substrates (six and 15 residues long) and the natural substrate, milk, revealed a reduction in the activity of the mutant enzyme with respect to the native when acting on both the long peptide substrate and milk. This may be a consequence of the different charge distribution of the mutated loop, its increased size and/or its different conformation.

- L35 ANSWER 40 OF 87 MEDLINE on STN DUPLICATE 15  
 97084792 Document Number: 97084792. PubMed ID: 8931126. Involvement of a residue at position 75 in the catalytic mechanism of a fungal aspartic proteinase, Rhizomucor pusillus pepsin. Replacement of tyrosine 75 on the flap by asparagine enhances catalytic efficiency. Park Y N; Aikawa J; Nishiyama M; Horinouchi S; Beppu T. (Department of Biotechnology, University of Tokyo, Japan. ) PROTEIN ENGINEERING, (1996 Oct) 9 (10) 869-75. Journal code: 8801484. ISSN: 0269-2139. Pub. country: ENGLAND: United Kingdom. Language: English.
- AB Residue 75 on the flap, a beta hairpin loop that partially covers the active site cleft, is tyrosine in most members of the aspartic proteinase family. **Site-directed mutagenesis** was carried out to investigate the functional role of this residue in Rhizomucor pusillus pepsin, an aspartic proteinase with high milk-clotting activity produced by the fungus Rhizomucor pusillus. A set of mutated enzymes with replacement of the amino acid at position 75 by 17 other amino acid residues except for His and Gly was constructed and their enzymatic properties were examined. Strong activity, higher than that of the wild-type enzyme, was found in the mutant with asparagine (Tyr75Asn), while weak but distinct activity was observed in Tyr75Phe. All the other mutants showed markedly decreased or negligible activity, less than 1/1000 of that of the wild-type enzyme. Kinetic analysis of Tyr75Asn using a chromogenic synthetic oligopeptide as a substrate revealed a marked increase in *k<sub>cat</sub>* with slight change in *K<sub>m</sub>*, resulting in a 5.6-fold increase in *k<sub>cat</sub>/K<sub>m</sub>*. When differential absorption spectra upon addition



of pepstatin, a specific inhibitor for aspartic proteinase, were compared between the wild-type and mutant enzymes, the wild-type enzyme and Tys75Asn, showing strong activity, had spectra with absorption maxima at 280, 287 and 293 nm, whereas the others, showing decreased or negligible activity, had spectra with only two maxima at 282 and 288 nm. This suggests a different mode of the inhibitor binding in the latter mutants. These observations suggest a crucial role of the residue at position 75 in enhancing the catalytic efficiency through affecting the mode of substrate-binding in the aspartic proteinases.

- L35 ANSWER 41 OF 87 MEDLINE on STN DUPLICATE 16  
 96313812 Document Number: 96313812. PubMed ID: 8713087. Involvement of the N-terminal part of cyclophilin B in the interaction with specific Jurkat T-cell binding sites. Mariller C; Haendler B; Allain F; Denys A; Spik G. (Laboratoire de Chimie Biologique, Universite des Sciences et Technologies de Lille, Villeneuve d'Ascq, France. ) BIOCHEMICAL JOURNAL, (1996 Jul 15) 317 ( Pt 2) 571-6. Journal code: 2984726R. ISSN: 0264-6021. Pub. country: ENGLAND: United Kingdom. Language: English.
- AB Cyclophilin B (CyPB) is secreted in biological fluids such as blood or milk and binds to a specific receptor present on the human lymphoblastic cell line Jurkat and on human peripheral blood lymphocytes. This study was intended to specify the areas of CyPB that are involved in the interaction with the receptor. A synthetic peptide corresponding to the first 24 N-terminal amino acid residues of CyPB was shown to specifically recognize the receptor. Moreover, modification of Arg18 of CyPB by p-hydroxyphenylglyoxal led to a dramatic loss of affinity for the receptor. However, when this residue was replaced by an alanine residue using **site-directed mutagenesis**, no modification of the binding properties was found, suggesting that Arg18 is not directly involved but is sufficiently close to the interaction site to interfere with the binding when modified. Competitive binding experiments using a chimeric protein made up of the 24 N-terminal amino acid residues of CyPB fused to the cyclophilin A core sequence confirmed the involvement of this region of CyPB in receptor binding.
- L35 ANSWER 42 OF 87 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
 1996:107171 Document No.: PREV199698679306. Study of putative glycosylation sites in bovine beta-casein introduced by PCR-based **site-directed mutagenesis**. Choi, Byung-Kwon; Jimenez-Flores, Rafael (1). (1) Dep. Dairy Sci., Calif. Polytechnic State Univ., San Luis Obispo, CA 93407 USA. Journal of Agricultural and Food Chemistry, (1996) Vol. 44, No. 1, pp. 358-364. ISSN: 0021-8561. Language: English.
- AB The bovine beta-casein gene, A-2 genetic variant, has been mutated at positions 70 and 71 for the introduction of a glycosylation signal (Asn-X-Ser). These mutants have been constructed to study the functionality of beta-casein glycosylated exclusively at Asn-68. The mutation was generated using PCR-based **site-directed mutagenesis**, and it was derived from bovine beta-casein cDNA. The mutant cDNAs including the wild-type beta-casein gene have been subcloned into the yeast *Pichia pastoris* expression vector pHIL-D2, which contains the methanol-inducible alcohol oxidase (AOX1) promoter. Three expression vectors were constructed and designated pCJ-beta-WT (wild-type bovine beta-casein gene), pCJ-beta-68 (substitution of Ser-70 for Leu-70), and pCJ-beta-6873 (Ser-70-Pro-71). Bovine beta-casein produced in yeast was found to contain a sugar moiety on Asn68 (N-linked glycosylated) when produced from a strain containing the pCJ-beta-6873 construct in its chromosome. N-Glycosylation of bovine beta-casein at position 68 was completely inhibited in transformants carrying vector pCJ-beta-68 with the single substitution of Ser-70 for Leu-70. The concentration of bovine beta-casein in this expression system was in the range of 0.7-1.0 g/L.
- L35 ANSWER 43 OF 87 MEDLINE on STN DUPLICATE 17  
 96314489 Document Number: 96314489. PubMed ID: 8706738. Recombinant soluble beta-1,4-galactosyltransferases expressed in *Saccharomyces cerevisiae*. Purification, characterization and comparison with human

enzyme. Malissard M; Borsig L; Di Marco S; Grutter M G; Kragl U; Wandrey C; Berger E G. (Institute of Physiology, University of Zurich, Switzerland. ) EUROPEAN JOURNAL OF BIOCHEMISTRY, (1996 Jul 15) 239 (2) 340-8. Journal code: 0107600. ISSN: 0014-2956. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB beta-1,4-Galactosyltransferase (Gal-T, EC 2.4.1.38) transfers galactose (Gal) from UDP-Gal to N-acetyl-D-glucosamine or a derivative GlcNAc-R. Soluble Gal-T, purified from human breast milk, was shown to be very heterogeneous by isoelectric focusing (IEF). In order to produce sufficient homogeneous enzyme for three-dimensional analysis, the human enzyme (hGal-T) has been expressed in *Saccharomyces cerevisiae*, production scaled up to 187 U recombinant Gal-T (rGal-T) and purified. The purification protocol was based on chromatography on concanavalin-A-Sepharose followed by affinity chromatographies on GlcNAc-Sepharose and alpha-lactalbumin-Sepharose. Analysis by SDS/PAGE revealed hyperglycosylation at the single N-glycosylation site, preventing recognition by antibodies. Analysis by IEF revealed considerable heterogeneity of rGal-T. The N-glycan could be removed by treatment with endoglycosidase H (endo H). The N-deglycosylated form of rGal-T retained full activity and showed only three isoforms by IEF analysis. Then we abolished the single N-glycosylation consensus sequence by **site-directed mutagenesis** changing Asn69-->Asp. The soluble mutated enzyme (N-deglycosylated rGal-T) was expressed in *S. cerevisiae* and its production scaled up to 60 U.N-deglycosylated rGal-T was purified to electrophoretic homogeneity. When analyzed by IEF, N-deglycosylated rGal-T was resolved in two bands. The O-glycans could be removed by jack bean alpha-mannosidase treatment and the completely deglycosylated Gal-T appeared homogeneous by IEF. The kinetic parameters of N-deglycosylated rGal-T were shown not to differ to any significant extent from those of the hGal-T. No significant changes in CD spectra were observed between hGal-T and N-deglycosylated rGal-T. Light-scattering analysis revealed dimerization of both enzymes. These data indicate that N-deglycosylated rGal-T was correctly folded, homogeneous and thus suitable for crystallization experiments.

L35 ANSWER 44 OF 87 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
96:693364 The Genuine Article (R) Number: VH069. MOLECULAR-BIOLOGY OF ENZYMES INVOLVED WITH CHOLESTEROL ESTER HYDROLYSIS IN MAMMALIAN-TISSUES. HUI D Y (Reprint). UNIV CINCINNATI, COLL MED, DEPT PATHOL & LAB MED, 231 BETHESDA AVE, CINCINNATI, OH, 45267 (Reprint). BIOCHIMICA ET BIOPHYSICA ACTA-LIPIDS AND LIPID METABOLISM (06 SEP 1996) Vol. 1303, No. 1, pp. 1-14. ISSN: 0005-2760. Pub. country: USA. Language: ENGLISH.

L35 ANSWER 45 OF 87 CAPLUS COPYRIGHT 2003 ACS on STN  
1996:113371 Document No. 124:173427 Arthritogenic intestinal flora replacement and method and vaccines for the treatment of rheumatoid arthritis. Carson, Dennis A.; Salvatore, Albani (Reagents of the University of California, USA). PCT Int. Appl. WO 9531984 A1 19951130, 51 pp. DESIGNATED STATES: W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TT, UA; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1995-US4896 19950424. PRIORITY: US 1994-246988 19940520.

AB Methods useful in the treatment or prevention of rheumatoid arthritis (RA) are disclosed. Each method is useful in limiting the exposure of the systemic immune system of a human to RA arthritogenic peptides present in the person's gastrointestinal (GI) tract. To this end, one method of the invention reduces the population of arthritogenic peptide-producing bacteria in the GI tract (e.g., by means of antibiotics) then replaces those bacteria with ones incapable of producing the arthritogenic peptides (e.g., bacteria altered by **site-directed mutagenesis** to express heat-shock protein dnaJ contg. the motif DERAAYDQYGHAAFE instead of QKRAAYDQYGHAAFE). Methods for both passive and

active immunization of a human against arthritogenic peptides are disclosed, as in a method for identifying persons who are predisposed to develop RA.

L35 ANSWER 46 OF 87 CAPLUS COPYRIGHT 2003 ACS on STN  
1995:643401 Document No. 123:49257 Inactivation of the hormone-responsive elements of a to prevent complications from ectopic expression gene therapy. Ebert, Karl M.; Ditullio, Paul; Cheng, Seng Hing; Meade, Harry M.; Smith, Alan Edward (Genzyme Corp., USA). PCT Int. Appl. WO 9510606 A1 19950420, 73 pp. DESIGNATED STATES: W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, UZ, VN; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG.  
(English). CODEN: P1XXD2. APPLICATION: WO 1994-US11504 19941011.  
PRIORITY: US 1993-135809 19931013.

AB Genes for use in gene therapy with inactivated hormone responsive element(s) are described. Inactivation of hormone-responsive elements is important to prevent hormone-induced expression of the gene at the wrong time or place or at excessive levels, leading to complications. The invention is aimed specifically at methods of treating subjects having cystic fibrosis that include administering a therapeutic gene to patients such that functional cystic fibrosis transmembrane conductance regulator is produced by the patient at a level that is not detrimental to the patient or to specific cell types. A method of assaying DNA for the presence or absence of a hormone-responsive element in a species in which the hormone responsive element is functional and a method of selectively breeding female transgenic mammals which produce a protein of interest. Expression constructs using the endogenous promoter region of the CFTR gene in combination with mammary gland-specific elements were used in the generation of transgenic rabbits. All of the male rabbits were either stillborn or died soon after birth. Synthesis of human CFTR in does with secretion of the protein into milk was observed. Androgen-responsive element-like sequences were found in the promoter region of the human CFTR gene and these were inactivated by site-directed mutagenesis. The use of androgen-responsive elements to drive ectopic expression of a gene leading to the death of male embryos is discussed.

L35 ANSWER 47 OF 87 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
95:254755 The Genuine Article (R) Number: QR526. STRUCTURE-FUNCTION ANALYSIS OF HUMAN ALPHA-1-3FUCOSYLTRANSFERASES - A GDP-FUCOSE-PROTECTED, N-ETHYLMALIMIDE-SENSITIVE SITE IN FUCT-III AND FUCT-V CORRESPONDS TO SER(178) IN FUCT-IV. HOLMES E H (Reprint); XU Z H; SHERWOOD A L; MACHER B A. PACIFIC NW RES FDN, 720 BROADWAY, SEATTLE, WA, 98122 (Reprint); SAN FRANCISCO STATE UNIV, DEPT CHEM & BIOCHEM, SAN FRANCISCO, CA, 94132. JOURNAL OF BIOLOGICAL CHEMISTRY (07 APR 1995) Vol. 270, No. 14, pp. 8145-8151. ISSN: 0021-9258. Pub. country: USA. Language: ENGLISH.  
\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Human alpha 1-->3fucosyltransferases constitute a family of closely related membrane-bound enzymes distinguished by differences in acceptor specificities and inherent protein biochemical properties. One such biochemical property is sensitivity to enzyme inactivation by sulphydryl-group modifying reagents such as N-ethylmaleimide. The basis for this property has been studied using a fusion protein of Fuct-III and Fuct-V composed of protein A coupled to the catalytic domain of the enzyme. The results indicate that modification of Fuct-V by 5,5'-dithiobis(2-nitrobenzoic acid) resulted in efficient enzyme inactivation that could be reversed by excess thiol reagent suggesting that the free sulphydryl group on the enzyme was required for activity. Recombinant forms of both Fuct-III and Fuct-V were irreversibly inactivated by N-ethylmaleimide and could be effectively protected from inactivation by GDP-fucose and GDP but not by UDP-galactose, fucose, or N-acetyllactosamine. Analysis of the distribution of Cys residues in aligned sequences of cloned human alpha 1-->3fucosyltransferases indicated

one site, Cys(143) of FucT-III and Cys(156) of FucT-V, corresponded to the highly conservative replacement of Ser(178) in FucT-IV, an enzyme insensitive to N-ethylmaleimide. A **site-directed mutagenesis** experiment was performed to replace Ser(178) of FucT-IV with a Cys residue. The mutant FucT-IV enzyme was active; however, the K-m for GDP-fucose was increased about 3-fold compared to the native enzyme to 28 +/- 3 mu M. This enzyme was N-ethylmaleimide sensitive and could be partially protected by GDP-fucose but not N-acetyllactosamine. These results support the importance of Ser(178) of FucT-IV in donor substrate binding and strongly suggest analogous Cys residues are the GDP-fucose protectable, N-ethylmaleimide-sensitive sites present in FucT-III and -V.

L35 ANSWER 48 OF 87 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN

95:235366 The Genuine Article (R) Number: QN899. RECOMBINANT HUMAN-MILK BILE-SALT-STIMULATED LIPASE - FUNCTIONAL-PROPERTIES ARE RETAINED IN THE ABSENCE OF GLYCOSYLATION AND THE UNIQUE PROLINE-RICH REPEATS. BLACKBERG L; STROMQVIST M (Reprint); EDLUND M; JUNEBLAD K; LUNDBERG L; HANSSON L; HERNELL O. SYMBICOM AB, TVISTEVAGEN 48, S-90736 UMEA, SWEDEN (Reprint); SYMBICOM AB, S-90736 UMEA, SWEDEN; UMEA UNIV, DEPT MED BIOCHEM & BIOPHYS, S-90187 UMEA, SWEDEN; ASTRA HASSLE AB, PRECLIN RES & DEV, MOLNDAL, SWEDEN; UMEA UNIV, DEPT PEDIAT, UMEA, SWEDEN. EUROPEAN JOURNAL OF BIOCHEMISTRY (15 MAR 1995) Vol. 228, No. 3, pp. 817-821. ISSN: 0014-2956. Pub. country: SWEDEN. Language: ENGLISH. \*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Human milk bile-salt-stimulated lipase ensures efficient utilization of milk lipid in breast-fed infants. The N-terminal two-thirds of the peptide chain is highly conserved and shows striking similarities to typical esterases. In contrast, the remaining C-terminal part consists of a unique sequence of 16 proline-rich O-glycosylated repeats of 11 residues each. Recently we could show, using recombinant Lipase variants, that neither these repeats nor the single N-linked sugar chain are essential for catalytic efficiency. In the present study, we report on the lack of importance of glycosylation and the unique repeats for other important functional properties, i.e. bile-salt activation, heparin binding, heat stability, stability at low pH and resistance to proteolytic inactivation. Compared to native enzyme, recombinant full-length lipase produced in two mammalian cell lines differed slightly in glycosylation pattern with no effects on the functional properties. Moreover, a variant lacking all repeats and the C-terminal tail following the last repeat exhibited the same functional characteristics as purified native milk enzyme. Thus, the structural basis for all the typical and functionally important properties reside in the N-terminal conserved part, in spite of the fact that none of these properties are shared by typical esterases. We could however, demonstrate that the C-terminal repeats are responsible for the unusual behaviour of the enzyme in size-exclusion chromatography, resulting in a considerably higher than expected apparent molecular mass.

L35 ANSWER 49 OF 87 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

1995:192841 Document No.: PREV199598207141. **Site-directed mutagenesis** of human milk bile salt-stimulated lipase (BSSL): Structure-function relations. Blackberg, L. (1); Edlund, M.; Hansson, L.; Juneblad, K.; Lundberg, L.; Stromqvist, M.; Hernell, O.. (1) Dep. Pediatr., Univ. Umea, Symbicom AB, Umea Sweden. FASEB Journal, (1995) Vol. 9, No. 3, pp. A184. Meeting Info.: Experimental Biology 95, Part I Atlanta, Georgia, USA April 9-13, 1995 ISSN: 0892-6638. Language: English.

L35 ANSWER 50 OF 87 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN

95:200394 The Genuine Article (R) Number: QJ987. **SITE-DIRECTED MUTAGENESIS OF HUMAN-MILK BILE SALT-STIMULATED LIPASE (BSSL)**. BLACKBERG L (Reprint); EDLUND M; HANSSON L; JUNEBLAD K; LUNDBERG L; STROMQVIST M; HERNELL O. UMEA UNIV, SYMBICOM AB, DEPT PEDIAT, UMEA, SWEDEN; UMEA UNIV, SYMBICOM AB, DEPT MED BIOCHEM & BIOPHYS, UMEA, SWEDEN; ASTRA HASSLE AB, R&D, MOLNDAL, SWEDEN. FASEB

- L35 ANSWER 51 OF 87 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN 94071208 EMBASE Document No.: 1994071208. YY1 represses .beta.-casein gene expression by preventing the formation of a lactation-associated complex. Raught B.; Khurshed B.; Kazansky A.; Rosen J.. Department of Cell Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, United States. Molecular and Cellular Biology 14/3 (1752-1763) 1994. ISSN: 0270-7306. CODEN: MCEBD4. Pub. Country: United States. Language: English. Summary Language: English.
- AB Site-specific **mutagenesis** of the highly conserved **milk** box (-140 to -110) region suggested that .beta.-casein expression is regulated by a hormone-mediated relief of repression (M. Schmitt-Ney, W. Doppler, R. K. Ball, and B. Groner, Mol. Cell. Biol. 11:3745-3755, 1991). However, when this sequence was placed upstream of a heterologous thymidine kinase promoter, it activated reporter gene expression. This apparent paradox was resolved when the trans-acting factor YY1, capable of acting as both a positive and negative regulator, was shown to interact with the **milk** box region, using bacterially expressed YY1 and specific oligonucleotide and antibody competition experiments. Second, it was demonstrated that extracts prepared from several cell types contained a protein(s) interacting with the mammary gland-specific factor (MGF) binding site, previously shown to be required for .beta.-casein promoter activity (Schmitt-Ney et al., Mol. Cell. Biol. 11:3745-3755, 1991). Sequence analysis of this site revealed similarity to the gamma interferon-activated sequence, suggesting that MGF may be related to the stat91 signaling protein. Finally, using an oligonucleotide encompassing both the YY1 and MGF sites, we detected a slow-mobility complex only in extracts from mammary glands at late pregnancy and lactation (lactation-associated complex [LAC]). Site-specific mutation of the YY1 binding site led to an enhancement in LAC DNA binding activity, while mutation of the MGF site decreased detectable LAC. These results support a model in which lactogenic stimuli lead to a decrease in YY1 binding, and subsequent increased formation of LAC at a nearby binding site, to stimulate .beta.-casein transcription.
- L35 ANSWER 52 OF 87 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN 94:701462 The Genuine Article (R) Number: PN634. IDENTIFICATION OF FUNCTIONAL CYSTEINE RESIDUES IN HUMAN GALACTOSYLTRANSFERASE. WANG Y; WONG S S; FUKUDA M N; ZU H Y; LIU Z D; TANG Q S; APPERT H E (Reprint). MED COLL OHIO, DEPT SURG, TOLEDO, OH, 43699 (Reprint); MED COLL OHIO, DEPT SURG, TOLEDO, OH, 43699; UNIV TEXAS, DEPT PATHOL & LAB MED, HOUSTON, TX, 77030; LA JOLLA CANC RES FDN, LA JOLLA, CA, 92037. BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS (28 OCT 1994) Vol. 204, No. 2, pp. 701-709. ISSN: 0006-291X. Pub. country: USA. Language: ENGLISH.
- \*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*
- AB The functions of the five cysteine residues in human galactosyltransferase were investigated using **site-directed mutagenesis** to determine the location of the disulfide bond as well as the role of the sulfhydryl groups. The enzyme remains active when three of its cysteine residues at positions 171, 264 and 340 are mutated to serine separately. However, enzymatic activity is lost when either cysteine-129 or cysteine-245 is replaced with serine. The loss of GT activity suggests that these two cysteine residues form a disulfide bond. The three active mutated enzymes were studied kinetically. The kinetic constants of the enzymes with cysteine-171 or cysteine-264 replaced with serine are not significantly different from those of GT that does not have these substitutions. When cysteine-340 was mutated, however, the kinetic constant for UDP-galactose increased about 30 fold, while that for N-acetylglucosamine and Mn<sup>2+</sup> remained unchanged. In addition, sulfhydryl inhibition studies reveal that cysteine-340 is the only cysteine residue that reacts with the sulfhydryl reagents. These results indicate that cysteine-340 may be involved in the binding of UDP-galactose. (C) 1994 Academic Press, Inc.

L35 ANSWER 53 OF 87 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
 94:588510 The Genuine Article (R) Number: PF401. RENNET - CURRENT TRENDS AND  
 FUTURE-RESEARCH. GARG S K (Reprint); JOHRI B N. AVADH UNIV, DEPT  
 MICROBIOL, FAIZABAD 224001, UTTAR PRADESH, INDIA (Reprint); GOVIND BALLABH  
 PANT UNIV AGR & TECHNOL, COLL BASIC SCI & HUMAN, DEPT MICROBIOL, PANTNAGAR  
 263145, UTTAR PRADESH, INDIA. FOOD REVIEWS INTERNATIONAL (1994) Vol. 10,  
 No. 3, pp. 313-355. ISSN: 8755-9129. Pub. country: INDIA. Language:  
 ENGLISH.

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB

A worldwide shortage of calf rennet for cheese production has existed for several decades. Bovine pepsin and to a certain extent porcine pepsin and plant coagulants have been used as rennet substitutes, but these have not been commercially successful owing to their extensive proteolytic nature and other inherent drawbacks. Investigations to develop other alternatives have resulted in the introduction of various microbial coagulants which have found markets in several countries despite certain shortcomings when compared with traditional calf rennet. However, in some countries, microbial coagulants have not been accepted for regular cheese manufacture because they are believed to result in a reduced yield and a lower quality product; this is particularly applicable to cheddar cheese manufacture.

Immobilization of proteases for milk coagulation has received renewed thrust; this may convert renneting of milk to a continuous operation. However, this technique has yet to be applied on an industrial scale; such a process is not likely to be successful since immobilized enzyme technology is dependent on the ability of a small, rapidly diffusing substrate to move quickly around the immobilized enzyme. In contrast, casein micelles are large and diffusion is slow, so the proteolysis rate is very slow. Microbial rennets offer an attractive target for genetic engineers since it may be possible to alter their structure/function characteristics to match those of calf rennet; application of **site-directed mutagenesis** could be particularly rewarding. The term 'calf rennet,' in the cheese industry, generally refers to an enzyme extract obtained from the fourth stomach (abomasum) of 10- to 30-day-old calves and used to coagulate **milk** for cheese production. The purified **milk-clotting** enzyme present in crude rennet preparations is known as 'rennin' or 'chymosin.' The designation 'chymosin' is now recommended in the international enzyme nomenclature (renin is associated with hypertension and is derived from the kidney). Calf rennet is referred to as 'animal rennet'; frequently, the term 'chymase' appears in the older literature as a synonym for rennet. In more general usage, however, any **milk-clotting** enzyme preparation yielding a relatively stable curd is designated as rennet. Christian Hansen is credited with the first industrial production of rennet in 1874 (1). This enzyme was called chymosin (EC 3.4.23.4); it belongs to the group of aspartic proteases and is the standard against which all other types of **milk-clotting** enzymes are compared. Since the origin of cheese production, the manufacturing process has always needed soluble enzymes to clot the **milk**; it has been adapted to the properties of calf rennet, an essential enzyme for this purpose. Rennet coagulates **milk** rapidly at its natural pH with little further degradation of the **milk** proteins. The zymogen, prorennin, is converted to chymosin at pH below 5.0, but optimally at pH 2.0 (2). Chymosin, a strong protease, has been crystallized (3) and detailed crystallographic studies of the enzyme have been reported by Bunn et al. (4). Besides a slightly high proteolytic activity, another disadvantage of this enzyme is that it is extracted from the abomasum of the unweaned calf. As the calf ages, chymosin is replaced by pepsin, although in cattle, the secretion of chymosin never comes to a complete stop. Although pepsin can clot **milk**, it has a tendency to result in higher fat losses because the curd formed has a more open, looser structure than that formed with chymosin; the cheese produced also has a softer body than desired. Pepsin and a commercial product under the trade name Metroclot (5, 6) have,

however, been used for the production of a variety of cheeses in the past. The kinetic properties and amino acid composition of chymosin and pepsin have been extensively studied. There is more or less a consensus of opinion in favor of chymosin as the enzyme for cheese making; presently three companies are producing calf chymosin through recombinant DNA technology. The cloned chymosin preparations produced by different microorganisms have been tested in various countries for cheese manufacture.

No major differences could be detected among cheeses made with cloned chymosin and those made with the natural enzyme. This review describes the past, present, and future status of rennet substitutes utilized as **milk** coagulants and applications of modern biological tools to strain improvement and process development.

L35 ANSWER 54 OF 87 CAPLUS COPYRIGHT 2003 ACS on STN

1994:211132 Document No. 120:211132 Analyses of structure-function relationship of practical enzymes and proteins by **site-directed mutagenesis**. Nishiyama, Makoto (Fac. Agric., Univ. Tokyo, Tokyo, 113, Japan). Nippon Nogei Kagaku Kaishi, 68(2), 205-11 (Japanese) 1994. CODEN: NNKKAA. ISSN: 0002-1407.

AB A review with 33 refs. on (1) protein engineering of thermostable malate dehydrogenase (MDH) including anal. of the thermophilic mechanism of tMDH by Thr-189 **mutagenesis** and conversion of the coenzyme specificity of tMDH by **site-directed mutagenesis**, (2) protein engineering of **milk-clotting** enzymes including the importance of Tyr-75 of these enzymes on the substrate specificity and conversion of amino acid residues in the substrate-binding subsites of the enzymes, and (3) protein engineering of Cu proteins including **site-directed mutagenesis** of nitrite dehydrogenase and pseudoazurin.

L35 ANSWER 55 OF 87 CAPLUS COPYRIGHT 2003 ACS on STN

1994:596381 Document No. 121:196381 The mammary factor MPBF is a prolactin-induced transcriptional regulator which binds to STAT factor recognition sites. Burdon, Thomas G.; Demmer, Jerome; Clark, A. John; Watson, Christine J. (Div. Mol. Biol., Roslin Inst., Roslin/Midlothian, EH25 9PS, UK). FEBS Letters, 350(2-3), 177-82 (English) 1994. CODEN: FEBLAL. ISSN: 0014-5793.

AB **Site-directed mutagenesis** of the three binding sites for the mammary factor MPBF in the .beta.-lactoglobulin (BLG) promoter demonstrate that MPBF is transcriptional activator of the BLG gene in mammary cells. MPBF requires phosphorylation of tyrosine for max. binding activity and binds to GAS (interferon .gamma.-activation site) elements which are similar to the MPBF binding sites. Prolactin induces MPBF binding activity in CHO cells and is not antigenically related to Stat1 (p91) and Stat2 (p113), suggesting that this transcription factor is likely to be another member of the STAT family of cytokine/growth factor-induced transcription factors.

L35 ANSWER 56 OF 87 MEDLINE on STN

DUPLICATE 18

94153527 Document Number: 94153527. PubMed ID: 7764448. Mutation of a fungal aspartic proteinase, Mucor pusillus rennin, to decrease thermostability for use as a **milk** coagulant. Yamashita T; Higashi S; Higashi T; Machida H; Iwasaki S; Nishiyama M; Beppu T. (Tokyo Research Laboratory, Meito Sangyo Co. Ltd., Japan. ) JOURNAL OF BIOTECHNOLOGY, (1994 Jan 15) 32 (1) 17-28. Journal code: 8411927. ISSN: 0168-1656. Pub. country: Netherlands. Language: English.

AB **Mutagenesis** of a fungus Mucor pusillus, a producer of an aspartic proteinase named Mucor pusillus rennin (MPR), was performed to obtain the mutated enzymes with decreased thermostability, which is desirable for practical use of the enzyme as a **milk** coagulant for cheese manufacturing. A fungal mutant strain which produced the mutant enzyme with distinctly reduced thermostability was isolated. Two different mutant alleles of the mpr gene, one with a single amino acid exchange of Ala101 for Thr and the other of Gly186 for Asp, were cloned

out of this mutant strain. The mutated mpr genes were expressed in *Saccharomyces cerevisiae* under the control of the yeast GAL7 promoter to produce the active enzymes in extracellular medium. Both of the mutations, especially Gly186Asp, were confirmed to cause a marked decrease in thermostability of the enzyme. All mutants possessing exchanges of Gly186 for various amino acids by **site-directed mutagenesis** showed a decrease in thermostability, indicating involvement of this residue to maintain a conformation of the enzyme. A double mutant having the both exchanges, Ala101Thr and Gly186Asp, in a single molecule showed the lowest thermostability without decrease in the enzymatic activity as well as the relative ratio of clotting to proteolytic activity.

- L35 ANSWER 57 OF 87 CAPLUS COPYRIGHT 2003 ACS ON STN  
 1994:433384 Document No. 121:33384 Protein structure/functionality in genetically modified **milk** proteins. Creamer, L K. (Food Sci. Sect., New Zealand Dairy Res. Inst., Palmerston North, N. Z.). Australasian Biotechnology, 4(1), 15-18 (English) 1994. CODEN: AUBIE5. ISSN: 1036-7128.
- AB A review with 19 refs. The relationship between protein structure and functionality in food proteins is being explored on a wide front. One of the techniques that is being brought to bear on this topic for dairy proteins is **site-directed mutagenesis** which can alter protein structure selectively and specifically. It is clear that to take max. advantage of these techniques an increased understanding of food protein functionality is needed. Paradoxically some of this greater understanding is coming from studies that use genetically-modified proteins. Manipulation of **milk** compn. by genetic means is also being considered seriously.
- L35 ANSWER 58 OF 87 CAPLUS COPYRIGHT 2003 ACS ON STN  
 1993:404020 Document No. 119:4020 A heat-labile *Mucor* rennet and its manufacture in yeast. Yamashita, Takashi; Higashi, Susumu; Higashi, Toshihiko; Machida, Haruo; Iwasaki, Shinjiro; Beppu, Teruhiko (Meito Sangyo Co., Ltd., Japan). Eur. Pat. Appl. EP 536770 A1 19930414, 37 pp. DESIGNATED STATES: R: CH, DE, FR, GB, IT, LI, NL. (English). CODEN: EPXDXD. APPLICATION: EP 1992-117279 19921009. PRIORITY: JP 1991-263878 19911011.
- AB A mutant of *Mucor* that synthesizes a rennet activity that is heat labile is prep'd. and the gene for the enzyme is cloned and expressed in yeast. The enzyme is useful in the processing of dairy products. *Mucor pusillus* IF04578(+) was mutagenized with nitrosoguanidine, plated on koji agar, and colonies screened for heat-labile proteinase activity. A Sau3A partial digest library in  $\lambda$ .EMBL3 from an isolate producing a heat-labile rennet was screened with a wild-type gene for the enzyme and the cloned gene placed under control of a yeast promoter. A series of amino acid substituted analogs of the enzyme were prep'd. by **site-directed mutagenesis** of the gene and the thermostability and enzymic properties of the enzymes manuf'd. in *Saccharomyces* studied. Analogs with substitutions at positions 101 and 186 were very heat-labile and had **milk** clotting/proteinase activity ratios of 0.55-1.08.
- L35 ANSWER 59 OF 87 MEDLINE on STN DUPLICATE 19  
 94075366 Document Number: 94075366. PubMed ID: 8253803. Recombinant human **milk** bile salt-stimulated lipase. Catalytic activity is retained in the absence of glycosylation and the unique proline-rich repeats. Hansson L; Blackberg L; Edlund M; Lundberg L; Stromqvist M; Hernell O. (Sybicom AB, Umea, Sweden.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1993 Dec 15) 268 (35) 26692-8. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.
- AB Human **milk** bile salt-stimulated lipase ensures efficient utilization of triacylglycerol by breast-fed infants. Cloning and sequencing of cDNA have revealed that the peptide chain consists of 722 amino acid residues showing only little homology to typical lipases. The sequence is identical to that of pancreatic carboxyl-ester hydrolase.



The COOH-terminal part contains 16 proline-rich repeats of 11 residues with O-linked carbohydrate. The only N-linked sugar chain is situated close to the active-site serine. Using C127 cells and a bovine papilloma virus vector, high and stable expression of full-length lipase and of several variants, obtained by **site-directed mutagenesis**, was achieved. The produced proteins were purified and further characterized. Variants lacking all, or all but two, repeats were active with similar specific activity and the same bile salt dependence as the native **milk** enzyme. Changing the asparagine necessary for N-glycosylation gave the same principal results. Active recombinant full-length lipase was also produced in a bacterial system. We conclude that neither glycosylation (N- or O-linked) nor the proline-rich repeats are essential for catalytic activity or bile salt activation of human **milk** bile salt-stimulated lipase.

L35 ANSWER 60 OF 87 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN 93:99391 The Genuine Article (R) Number: KM161. **SITE-DIRECTED MUTAGENESIS OF A PUTATIVE HEPARIN BINDING**

DOMAIN OF AVIAN LIPOPROTEIN-LIPASE. BERRYMAN D E; BENSADOUN A (Reprint). CORNELL UNIV, DIV NUTR SCI, 321 SAVAGE HALL, ITHACA, NY, 14853; CORNELL UNIV, DIV BIOL SCI, ITHACA, NY, 14853. JOURNAL OF BIOLOGICAL CHEMISTRY (15 FEB 1993) Vol. 268, No. 5, pp. 3272-3276. ISSN: 0021-9258. Pub. country: USA. Language: ENGLISH.

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Lipoprotein lipase (LPL) binds to heparin and heparan sulfate proteoglycans. We have employed **site-directed mutagenesis** to dissect one of the proposed heparin binding domains of avian LPL, which contains the sequence Arg-Lys-Asn-Arg (amino acids 281-284). Various single, double, and triple mutants of chicken LPL were constructed in order to alter the positive charge of this region. The mutant and wild-type cDNAs were subcloned into an expression vector, pRc/CMV, and expressed in Chinese hamster ovary cells. In general, the LPL mutants with a decrease in regional positive charge showed a decrease in affinity for heparin and heparan sulfate proteoglycans. The greatest effect was seen with the triple mutant, LPL 5G, in which all of the positively charged amino acids were altered to neutral residues. On a heparin-Sepharose column, LPL 5G eluted at 0.96 M NaCl compared with 1.35 M for wild-type LPL. This mutant also had the lowest specific activity with 1.5 mueq fatty acid/mug/h for the cell-associated pool and with no detectable activity in the media. Wild-type cells, however, produced a lipase with a specific activity of 12.4 and 13.1 mueq fatty acid/mug/h for cell-associated and media lipase pools, respectively. LPL 5G also showed a decrease in affinity for the heparan sulfate proteoglycans on the cell surface of Chinese hamster ovary cells. In conclusion, the region of avian LPL between Arg281 and Arg284 does appear to be involved in heparin-binding; however, additional regions must be involved since binding was not completely abolished. In addition, specific activity of the cell-associated and secreted LPL is correlated to affinity of the enzyme for heparan sulfate chains.

L35 ANSWER 61 OF 87 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN 93:489920 The Genuine Article (R) Number: LQ598. FUNCTIONAL-CHARACTERIZATION OF A CHIMERIC LIPASE GENETICALLY-ENGINEERED FROM HUMAN LIPOPROTEIN-LIPASE AND HUMAN HEPATIC LIPASE. DICHEK H L (Reprint); PARROTT C; RONAN R; BRUNZELL J D; BREWER H B; SANTAMARINA-FOJO S. NHLBI, MOLEC DIS BRANCH, BLDG 10-7N117, 9000 ROCKVILLE PIKE, BETHESDA, MD, 20892 (Reprint); UNIV WASHINGTON, DEPT MED, DIV METAB ENDOCRINOL & NUTR, SEATTLE, WA, 98195. JOURNAL OF LIPID RESEARCH (AUG 1993) Vol. 34, No. 8, pp. 1393-1401. ISSN: 0022-2275. Pub. country: USA. Language: ENGLISH.

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Lipoprotein lipase (LPL) and hepatic lipase (HL) mediate the hydrolysis of triglycerides and phospholipids present in circulating lipoprotein particles and are essential for normal lipid metabolism. Both enzymes have a similar primary amino acid structure and share requirements for intact catalytic, lipid binding, and heparin binding domains. However, LPL and HL

exhibit different substrate specificities and cofactor requirements. In order to characterize the functional domains necessary for LPL activity, a chimeric lipase consisting of the amino-terminal 314 amino acids of human LPL and the carboxyl-terminal 146 amino acids of human HL was synthesized by joining the cDNA of both lipases at the 5'-end of exon 7. Northern blot hybridization and Western blot analyses revealed the size of the chimera mRNA and protein to be approximately 1.5 kb and 55 kDa, respectively. The chimeric enzyme hydrolyzed both long chain and short chain fatty acid triacylglycerols and had catalytic properties that were similar to lipoprotein lipase. Thus, apolipoprotein (apo)C-II was required for maximal lipase activity, and high salt concentration abolished the ability of the chimera to hydrolyze triolein even in the presence of apoC-II. A monospecific anti-HL polyclonal antibody interacting with the C-terminal HL-derived domain of the chimeric enzyme abolished the enzyme's ability to hydrolyze triglyceride emulsion but not tributyrin substrates. Analysis of the heparin binding properties of the chimeric enzyme using heparin-Sepharose affinity chromatography revealed an elution pattern which was intermediate between that of lipoprotein and hepatic lipase. In summary, we have characterized the functional properties of an LPL-HL chimeric enzyme. Our studies indicate that the LPL-derived NH2-terminal domain is the site of interaction between apoC-II and LPL and determines the catalytic properties of the chimera, whereas the HL-derived C-terminal domain plays a major role in mediating the lipase interaction with long chain triacylglycerol substrates. In addition, both the LPL-derived NH2-terminal domain and the HL-derived C-terminal domain contribute to the heparin binding properties of the LPL-chimera.

L35 ANSWER 62 OF 87 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN 93285291 EMBASE Document No.: 1993285291. Roles of extracellular and cytoplasmic domains of the prolactin receptor in signal transduction to milk protein genes. Lesueur L.; Edery M.; Paly J.; Kelly P.A.; Djiane J.. Endocrinologie Moléculaire, INRA, 78352 Jouy-en-Josas Cedex, France. Molecular Endocrinology 7/9 (1178-1184) 1993. ISSN: 0888-8809. CODEN: MOENEN. Pub. Country: United States. Language: English. Summary Language: English.

AB We have previously shown that the long form of the PRL receptor is able to activate milk protein gene transcription. In the present study, we have determined the respective contribution of the extracellular and the intracellular domains of this receptor to transcriptional activation of a milk protein gene by PRL. The membrane-anchored intracellular domain (pTMI) expressed alone was devoid of PRL binding activity, as expected, and did not constitutively stimulate expression of the target gene. The extracellular domain (pE), expressed alone as a soluble receptor form, binds PRL with 10- fold higher affinity than the full-length membrane receptor. This form was also unable to stimulate the expression of the reporter gene. However, expression of both mutants (pE + pTMI) in the same cell partially restored the ability of PRL to activate the .beta.-lactoglobulin promoter. Replacement of cysteine 184 by a serine in the extracellular domain of the receptor impairs this restoration of the biological response. However, introduction of the same mutation in the full-length receptor did not affect its functional activity. These results indicate that the membrane-anchored cytoplasmic domain of the PRL receptor has no constitutive activity, and that coexpression of individual extracellular and intracellular domains leads to restoration of receptor function. We propose that restoration may be the result of reconstitution of the holoreceptor through disulfide bonding, or it may be the result of interaction of the extracellular region with an external transducing molecule.

L35 ANSWER 63 OF 87 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN 93:178638 The Genuine Article (R) Number: KR461. LACK OF HORMONE BINDING IN COS-7 CELLS EXPRESSING A MUTATED GROWTH-HORMONE RECEPTOR FOUND IN LARON DWARFISM. EDERY M; ROZAKISADCOCK M; GOUJON L; FINIDORI J; LEVIMEYRUEIS C; PALY J; DJIANE J; POSTELVINAY M C; KELLY P A (Reprint). FAC NECKER ENFANTS MALAD, INSERM, U344, F-75015 PARIS, FRANCE; INRA, UNITE ENDOCRINOL MOLEC,

F-78352 JOUY EN JOSAS, FRANCE; MCGILL UNIV, ROYAL VICTORIA HOSP, MOLEC ENDOCRINOL LAB, MONTREAL H3A 1A1, QUEBEC, CANADA. JOURNAL OF CLINICAL INVESTIGATION (MAR 1993) Vol. 91, No. 3, pp. 838-844. ISSN: 0021-9738. Pub. country: FRANCE; CANADA. Language: ENGLISH.  
 \*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

- AB A single point mutation in the growth hormone (GH) receptor gene generating a Phe --> Ser substitution in the extracellular binding domain of the receptor has been identified in one family with Laron type dwarfism. The mutation was introduced by **site-directed mutagenesis** into cDNAs encoding the full-length rabbit GH receptor and the extracellular domain or binding protein (BP) of the human and rabbit GH receptor, and also in cDNAs encoding the full length and the extracellular domain of the related rabbit prolactin (PRL) receptor. All constructs were transiently expressed in COS-7 cells. Both wild type and mutant full-length rabbit GH and PRL receptors, as well as GH and prolactin BPs (wild type and mutant), were detected by Western blot in cell membranes and concentrated culture media, respectively. Immunofluorescence studies showed that wild type and mutant full-length GH receptors had the same cell surface and intracellular distribution and were expressed with comparable intensities. In contrast, all mutant forms (full-length receptors or BPs), completely lost their ability to bind ligand. These results clearly demonstrate that this point mutation (patients with Laron syndrome) does not modify the synthesis or the intracellular pathway of receptor proteins, but rather abolishes ability of the receptor or BP to bind GH and is thus responsible for the extreme GH resistance in these patients.

L35 ANSWER 64 OF 87 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN 1993:367005 Document No.: PREV199396052680. Field evaluation of the deosan-RMTK, a diagnostic test for the detection of dairy cows with high somatic cell count. Noordhuizen, J. P. T. M. (1); Stassen, E. N.; Klerx, I. (1). (1) Landbouw Univ. Wageningen, Vakgroep Veehouderij, Sectie Gezondheidsleer Reprod., Postbus 338, 6700 AH Wageningen Netherlands Antilles. Tijdschrift voor Diergeneeskunde, (1993) Vol. 118, No. 10, pp. 329-331. ISSN: 0040-7453. Language: Dutch. Summary Language: Dutch; English.

- AB The Deosan-Rapid Mastitis Test Kit (RMTK) was evaluated in 226 lactating dairy cows on 6 farms. The Fossomatic method was used as reference standard for somatic cell counts in cows milk. The RMTK test-principle regards the reaction of the enzyme catalase released from cells in milk with the H-2O-2 in the RMTK-reagent. For the threshold cellcount values of 250.000, 400.000 and 800.00/ml the following 95% confidence intervals were found: sensitivity 0.60-0.99, specificity 0.42-0.83, predictive value positive 0.22-0.46, predictive value negative 0.84-0.99 and kappa-value 0.14-0.52. Because this test will be most useful when the positive predictive value would be high, it is concluded that the RMTK in this study population was not an adequate tool for the detection of cows with somatic cell counts over 250.000/ml milk.

L35 ANSWER 65 OF 87 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN 1993:317118 Document No.: PREV199396025468. Determination of ceftiofur and its metabolite defuroylceftiofur in bovine serum and milk by ion-paired liquid chromatography. Tyczkowska, Krystyna L. (1); Voyksner, Robert D.; Anderson, Kevin L.; Aronson, Arthur L. (1) Clinical Pharmacol. Unit, Dep. Anatomy, Physiol. Sci. Radiol., North Carolina State Univ., 4700 Hillsborough St., Raleigh, NC 27606 USA. Journal of Chromatography Biomedical Applications, (1993) Vol. 614, No. 1, pp. 123-134. Language: English.

- AB A simple and sensitive liquid chromatographic method has been developed for the simultaneous determination of ceftiofur and its metabolite defuroylceftiofur in bovine serum and milk. The method involved an ultrafiltration of diluted serum/milk with an equal volume of 50% acetonitrile through a 10 000 dalton molecular mass cut-off filter. Separation of ceftiofur and defuroylceftiofur from the other serum/milk components was performed by ion-paired (octane and

dodecanesulfonate) liquid chromatography using a reversed-phase column eluted with acetonitrile-water solution. The ultraviolet-visible absorbance of the column effluent was monitored in 200-350 nm range of a photodiode-array detector or at lambda-max 289.6 nm for ceftiofur, lambda-max 265.8 nm for desfuroylceftiofur and lambda-max 271.4 nm for dimer of desfuroylceftiofur. Recoveries of ceftiofur from bovine milk spiked with 1 and 10 mu-g/ml were 95.9 and 97.0% with coefficients of variation of 3.69 and 2.51%, respectively. Recovery of ceftiofur from bovine serum spiked with 10 mu-g/ml was 90.4% with a coefficient of variation of 5.29%. A correlation coefficient of 0.9992 occurred with ceftiofur in aqueous solutions (n = 5, in duplicates). The limit of detection was estimated to be approximately 50 ppb (ng/ml). Additionally, this paper documents the presence of a ceftiofur metabolite in bovine serum under in vitro and in vivo conditions. The metabolite was identified as desfuroylceftiofur together with its dimer 3,3'-desfuroylceftiofur disulfide by thermospray liquid chromatography-mass spectrometry.

L35 ANSWER 66 OF 87 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
92:220839 The Genuine Article (R) Number: HL679. XANTHINE DEHYDROGENASE FROM DROSOPHILA-MELANOGASTER - PURIFICATION AND PROPERTIES OF THE WILD-TYPE ENZYME AND OF A VARIANT LACKING IRON SULFUR CENTERS. HUGHES R K; BENNETT B; BRAY R C (Reprint). UNIV SUSSEX, SCH BIOL SCI, BIOCHEM LAB, BRIGHTON BN1 9QG, E SUSSEX, ENGLAND. BIOCHEMISTRY (31 MAR 1992) Vol. 31, No. 12, pp. 3073-3083. ISSN: 0006-2960. Pub. country: ENGLAND. Language: ENGLISH. \*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Xanthine dehydrogenase has been purified to homogeneity by conventional procedures from the wild-type strain of the fruit fly *Drosophila melanogaster*, as well as from a rosy mutant strain (E89 --> K, ry5231) known to carry a point mutation in the iron-sulfur domain of the enzyme. The wild-type enzyme had all the specific properties that are peculiar to the molybdenum-containing hydroxylases. It had normal contents of molybdenum, the pterin molybdenum cofactor, FAD, and iron-sulfur centers. EPR studies showed its molybdenum center to be quite indistinguishable from that of milk xanthine oxidase. As isolated, only about 10% of the enzyme was present in the functional form, with most or all of the remainder as the inactive desulfo form. It is suggested that this may be present in vivo. Extensive proteolysis accompanied by the development of oxidase activity took place during isolation, but dehydrogenase activity was retained. EPR properties of the reduced iron-sulfur centers, Fe-SI and Fe-SII, in the enzyme are very similar to those of the corresponding centers in milk xanthine oxidase. The E89 --> K mutant enzyme variant was in all respects closely similar to the wild-type enzyme, with the exception that it lacked both of the iron-sulfur centers. This was established both by its having the absorption spectrum of a simple flavoprotein and by the complete absence of EPR signals characteristic of iron-sulfur centers in the reduced enzyme. Despite the lack of iron-sulfur centers, the mutant enzyme had xanthine:NAD+ oxidoreductase activity indistinguishable from that of the wild-type enzyme. Stopped-flow measurements indicated that, as for the wild-type enzyme, reduction of the mutant enzyme was rate-limiting in turnover. Thus, the iron-sulfur centers appear irrelevant to the normal turnover of the wild-type enzyme with these substrates. However, activity to certain oxidizing substrates, particularly phenazine methosulfate, is abolished in the mutant enzyme variant. This is one of the first examples of deletion by genetic means of iron-sulfur centers from an iron-sulfur protein. The relevance of our findings both to the roles of iron-sulfur centers in other systems and to the nature of the oxidizing substrate for the *Drosophila* enzyme in vivo are briefly discussed.

L35 ANSWER 67 OF 87 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STNDUPLICATE  
20

92355885 EMBASE Document No.: 1992355885. Protein engineering of the milk-clotting aspartic proteinases. Aikawa J.; Nishiyama M.; Beppu T. Department of Agricultural Chemistry, University of Tokyo, Yayoi

1-1-1, Bunkyo-ku, Tokyo 113, Japan. Scandinavian Journal of Clinical and Laboratory Investigation, Supplement 52/210 (51-58) 1992.  
ISSN: 0085-591X. CODEN: SCLSAH. Pub. Country: United Kingdom. Language: English. Summary Language: English.

- AB Calf Chymosin and a fungal protease from *Mucor pusillus* (*Mucor rennin*) are members of the aspartic proteinases used as **milk-coagulants** in cheese industry. A system for production of recombinant chymosin as inclusion bodies in *Escherichia coli* cells and its refolding into the active form was established. Another expression system for production of *Mucor rennin* in *Saccharomyces cerevisiae* was also established. *Mucor rennin* was efficiently excreted from the yeast host as a heavily glycosylated form. Glycosylation affected both the secretion and the enzyme properties. **Site-directed mutagenesis** of the Tyr residue at position 75 in chymosin and *Mucor rennin* revealed its crucial role in catalytic function of the aspartic proteinases. The results also suggested the possibility to improve practical properties of the **milk-clotting enzymes by site-directed mutagenesis**.

L35 ANSWER 68 OF 87 MEDLINE on STN

93088004 Document Number: 93088004. PubMed ID: 1455180. Protein engineering of the **milk-clotting aspartic proteinases**. Aikawa J; Nishiyama M; Beppu T. (Department of Agricultural Chemistry, University of Tokyo, Japan. ) SCANDINAVIAN JOURNAL OF CLINICAL AND LABORATORY INVESTIGATION. SUPPLEMENT, (1992) 210 51-8. Ref: 24. Journal code: 2984789R. ISSN: 0085-591X. Pub. country: Norway. Language: English.

- AB Calf Chymosin and a fungal protease from *Mucor pusillus* (*Mucor rennin*) are members of the aspartic proteinases used as **milk-coagulants** in cheese industry. A system for production of recombinant chymosin as inclusion bodies in *Escherichia coli* cells and its refolding into the active form was established. Another expression system for production of *Mucor rennin* in *Saccharomyces cerevisiae* was also established. *Mucor rennin* was efficiently excreted from the yeast host as a heavily glycosylated form. Glycosylation affected both the secretion and the enzyme properties. **Site-directed mutagenesis** of the Tyr residue at position 75 in chymosin and *Mucor rennin* revealed its crucial role in catalytic function of the aspartic proteinases. The results also suggested possibility to improve practical properties of the **milk-clotting enzymes by site-directed mutagenesis**.

L35 ANSWER 69 OF 87 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN

92:686221 The Genuine Article (R) Number: JY988. PROTEIN ENGINEERING OF THE **MILK-CLOTTING ASPARTIC PROTEINASES**. AIKAWA J; NISHIYAMA M; BEPPU T (Reprint). UNIV TOKYO, DEPT AGR CHEM, YAYOI 1-1-1, BUNKYO KU, TOKYO 113, JAPAN. SCANDINAVIAN JOURNAL OF CLINICAL & LABORATORY INVESTIGATION (1992) Vol. 52, Supp. 210, pp. 51-58. ISSN: 0036-5513. Pub. country: JAPAN. Language: ENGLISH.

- \*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*  
AB Calf Chymosin and a fungal protease from *Mucor pusillus* (*Mucor rennin*) are members of the aspartic proteinases used as **milk-coagulants** in cheese industry. A system for production of recombinant chymosin as inclusion bodies in *Escherichia coli* cells and its refolding into the active form was established. Another expression system for production of *Mucor rennin* in *Saccharomyces cerevisiae* was also established. *Mucor rennin* was efficiently excreted from the yeast host as a heavily glycosylated form. Glycosylation affected both the secretion and the enzyme properties. **Site-directed mutagenesis** of the Tyr residue at position 75 in chymosin and *Mucor rennin* revealed its crucial role in catalytic function of the aspartic proteinases. The results also suggested possibility to improve practical properties of the **milk-clotting enzymes by site-directed mutagenesis**.

L35 ANSWER 70 OF 87 CAPLUS COPYRIGHT 2003 ACS on STN

- 1991:241389 Document No. 114:241389 Somatotropin analogs with reduced hydrophobicity or helical stability for enhancing animal growth and milk production. Lehrman, Sherwood Russ; Havel, Henry A.; Tuls, Jody L.; Plaisted, Scott M.; Brems, David N. (Upjohn Co., USA). PCT Int. Appl. WO 9100870 A1 19910124, 24 pp. DESIGNATED STATES: W: AU, BB, BG, BR, CA, FI, HU, JP, KP, KR, LK, MC, MG, MW, NO, RO, SD, SU, US; RW: AT, BE, BF, BJ, CF, CG, CH, CM, DE, DK, ES, FR, GA, GB, IT, LU, ML, MR, NL, SE, SN, TD, TG. (English). CODEN: PIXD2. APPLICATION: WO 1990-US3550 19900627. PRIORITY: US 1989-377926 19890710.
- AB Animal somatotropins having single or multiple changes in amino acid residues corresponding to bovine somatotropin (bST) residues 96-133 are described. These analogs have reduced hydrophobicity or helical stability and thus self-assoc. or aggregate less than the parent proteins or have less potential for hydrophobic interactions or partial denaturation. The analogs are useful for enhancing animal growth and milk prodn. (Gln-121) bST (I) and (Leu-112 + Gln-121) bST (II) were obtained by oligonucleotide-directed **mutagenesis** using the oligonucleotide 5'-GAAGGCCAGGCTTGATGC-3' on the wild-type DNA sequence and on the Leu-112 DNA sequence, resp. Analog (Gly-125 + Arg-126) bST (III) was also prepd. I and III pptd. less (36 and 72% less, resp.) than wild-type bST; II pptd. more. The changes in I and II destabilized, while the changes in II stabilized, the assoc. folding intermediate. At low protein concns., I and III refold at the same rate as wild-type bST but at high protein conc. they fold faster while II refolds slower. Cows injected i.m. with I or (Leu-112) bST had slightly increased and reduced, resp., milk prodn.
- L35 ANSWER 71 OF 87 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. ON STN 91269578 EMBASE Document No.: 1991269578. Biosynthesis of glycosylated human lysozyme mutants. Horst M.; Harth N.; Hasilik A.. Inst. of Physiological Chem., Pathobiochemistry, Waldeyerstr. 15,D-4400 Munster, Germany. Journal of Biological Chemistry 266/21 (13914-13919) 1991. ISSN: 0021-9258. CODEN: JBCHA3. Pub. Country: United States. Language: English. Summary Language: English.
- AB Complementary DNA encoding human lysozyme was subjected to oligonucleotide-directed **mutagenesis**. At one of three selected positions, amino acid residues 22, 68, or 118, the signal for N-linked glycosylation was created. The mutant DNAs were inserted into a eucaryotic vector and transfected into cultured hamster cells. The three mutant cDNAs directed synthesis of lysozyme mutants, which were named LI, LII, and LIII. The mutant lysozymes LI and LII comprised mixtures of glycosylated and nonglycosylated forms. The glycosylated and nonglycosylated forms of mutant LI were found to have an enzymatic activity similar to normal human milk lysozyme. The usage of the glycosylation sites in the mutants was similar in Chinese hamster ovary (CHO) and baby hamster kidney cells. Approximately two of every three molecules in mutant LI, approximately one of every eight molecules in mutant LII, and practically no molecules in mutant LIII became glycosylated. In CHO cells, the processing of the oligosaccharide side chains yielded several larger products than in baby hamster kidney cells. This size variability of glycosylated lysozyme from CHO cells may be explained by the presence of biantennary and triantennary endo-.beta.-N-acetylglucosaminidase H-resistant oligosaccharides with N-acetylglucosamine repeats of variable length and by the presence of hybrid oligosaccharides, as suggested by affinity to several lectins and sensitivity to endo-.beta.-galactosidase. In both cell types, the majority of the glycosylated forms were secreted and thus behaved similarly to nonglycosylated lysozyme. A small proportion of mutant LI lysozyme remained associated with the cells. The retained lysozyme was recruited predominantly from the molecules bearing high mannose oligosaccharides. These molecules were targeted to lysosomes, and their carbohydrate was trimmed to an endo-.beta.-N-acetylglucosaminidase H-resistant form. Owing to the small size of mutant LI lysozyme, minor changes in the size of its carbohydrate moiety result in detectable changes in the electrophoretic mobility of the whole glycoprotein. We suggest that this novel glycoprotein could be used as a reporter in studies on processing and

segregation of glycoproteins.

L35 ANSWER 72 OF 87 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN  
91305087 EMBASE Document No.: 1991305087. Coregulation of the *Kluyveromyces*  
*lactis* lactose permease and .beta.-galactosidase genes is achieved by  
interaction of multiple LAC9 binding sites in a 2.6 kbp divergent  
promoter. Godecke A.; Zachariae W.; Arvanitidis A.; Breunig K.D..  
Institute of Microbiology, Heinrich-Heine-Universität Düsseldorf, 4000 Düsseldorf  
1, Germany. Nucleic Acids Research 19/19 (5351-5358) 1991.  
ISSN: 0305-1048. CODEN: NARHAD. Pub. Country: United Kingdom. Language:  
English. Summary Language: English.

AB The coregulated genes LAC4 and LAC12 encoding .beta.-galactosidase and  
lactose permease, respectively, are responsible for the ability of the  
milk yeast *Kluyveromyces lactis* to utilise lactose. They are  
divergently transcribed and separated by an unusually large intergenic  
region of 2.6 kbp. Mapping of the upstream border of the  
.beta.-galactosidase gene (LAC4) promoter by introduction of mutations at  
the chromosomal locus showed that LAC4 and LAC12 share the same upstream  
activation sites (UAS). The UASs represent binding sites for the  
trans-activator LAC9, a *K.lactis* homologue of GAL4, conforming to the  
consensus sequence 5'-CGG(N5)A/T(N5)CCG-3'. Two binding sites are located  
in front of each of the genes at almost symmetrical positions.  
.beta.-galactosidase activity measurements as well as quantitation of LAC4  
and LAC12 mRNA levels demonstrated that all four sites are required for  
full induction. LAC4 proximal and a LAC12 proximal sites cooperate in  
activating transcription of both genes. These sites are more than 1.7 kbp  
apart and the distal site is located more than 2.3 kbp upstream of the  
respective start of transcription. Thus, the distance between interacting  
sites is larger than in any of the well characterised yeast promoters. The  
contribution to gene activation differs for individual binding sites and  
correlates with the relative affinity of LAC9 for these sites in vitro  
suggesting that LAC9 binding is a rate limiting step for LAC promoter  
function.

L35 ANSWER 73 OF 87 MEDLINE on STN  
91294198 Document Number: 91294198. PubMed ID: 1906066. Lactococcal  
proteinase maturation protein PrtM is a lipoprotein. Haandrikman A J; Kok  
J; Venema G. (Department of Genetics, University of Groningen, Haren, The  
Netherlands. ) JOURNAL OF BACTERIOLOGY, (1991 Jul) 173 (14) 4517-25.  
Journal code: 2985120R. ISSN: 0021-9193. Pub. country: United States.  
Language: English.

AB The production of enzymatically active proteinase by lactococci requires  
the joint presence of a proteinase gene, prtP, and a gene encoding a  
maturation protein, prtM. A 32-kDa protein produced by *Escherichia coli*  
upon expression of the prtM gene under the direction of the T7 RNA  
polymerase promoter was purified and used to obtain PrtM-specific  
antibodies. With these antibodies, immunogold labeling of lactococcal  
cells revealed that PrtM was associated with the lactococcal cell  
envelope. Western blot (immunoblot) analysis of whole lactococcal cells  
and isolated membrane vesicles indicated that PrtM was a  
membrane-associated protein. Radiolabeling of *Lactococcus lactis* with  
[3H]palmitic acid showed that PrtM was a lipoprotein. Partial secretion  
of PrtM into the culture medium was observed after Cys-24, the target  
residue for lipid modification, was replaced by an Ala residue by means of  
site-directed mutagenesis. This mutation did  
not affect proteinase activity.

L35 ANSWER 74 OF 87 MEDLINE on STN  
91286196 Document Number: 91286196. PubMed ID: 1905714. A highly  
thermostable neutral protease from *Bacillus caldolyticus*: cloning and  
expression of the gene in *Bacillus subtilis* and characterization of the  
gene product. van den Burg B; Enequist H G; van der Haar M E; Eijlsink V G;  
Stulp B K; Venema G. (Department of Genetics, Centre of Biological  
Sciences, Haren, The Netherlands. ) JOURNAL OF BACTERIOLOGY, (1991 Jul)  
173 (13) 4107-15. Journal code: 2985120R. ISSN: 0021-9193. Pub. country:

United States. Language: English.

AB By using a gene library of *Bacillus caldolyticus* constructed in phage lambda EMBL12 and selecting for proteolytically active phages on plates supplemented with 0.8% skim milk, chromosomal B. caldolyticus DNA fragments that specified proteolytic activity were obtained. Subcloning of one of these fragments in a protease-deficient *Bacillus subtilis* strain resulted in protease proficiency of the host. The nucleotide sequence of a 2-kb *Hinf*I-*Mlu*I fragment contained an open reading frame (ORF) that specified a protein of 544 amino acids. This ORF was denoted as the B. caldolyticus npr gene, because the nucleotide and amino acid sequences of the ORF were highly similar to that of the *Bacillus stearothermophilus* npr gene. Additionally, the size, pH optimum, and sensitivity to the specific Npr inhibitor phosphoramidon of the secreted enzyme indicated that the B. caldolyticus enzyme was a neutral protease. The B. stearothermophilus and B. caldolyticus enzymes differed at only three amino acid positions. Nevertheless, the thermostability and optimum temperature of the B. caldolyticus enzyme were 7 to 8 degrees C higher than those of the B. stearothermophilus enzyme. In a three-dimensional model of the B. stearothermophilus Npr the three substitutions (Ala-4 to Thr, Thr-59 to Ala, and Thr-66 to Phe) were present at solvent-exposed positions. The role of these residues in thermostability was analyzed by using **site-directed mutagenesis**. It was shown that all three amino acid substitutions contributed to the observed difference in thermostability between the neutral proteases from B. stearothermophilus and B. caldolyticus.

L35 ANSWER 75 OF 87 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN 91:388038 The Genuine Article (R) Number: FV039. A HIGHLY THERMOSTABLE NEUTRAL PROTEASE FROM *BACILLUS-CALDOLYTICUS* - CLONING AND EXPRESSION OF THE GENE IN *BACILLUS-SUBTILIS* AND CHARACTERIZATION OF THE GENE-PRODUCT. VANDENBURG B (Reprint); ENEQUIST H G; VANDERHAAR M E; EIJSINK V G H; STULP B K; VENEMA G. CTR BIOL SCI, DEPT GENET, KERKLAAN 30, 9751 NN HAREN, NETHERLANDS (Reprint); STATE UNIV GRONINGEN, DEPT BIOCHEM, 9747 AG GRONINGEN, NETHERLANDS. JOURNAL OF BACTERIOLOGY (1991) Vol. 173, No. 13, pp. 4107-4115. Pub. country: NETHERLANDS. Language: ENGLISH. \*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB By using a gene library of *Bacillus caldolyticus* constructed in phage lambda EMBL12 and selecting for proteolytically active phages on plates supplemented with 0.8% skim milk, chromosomal B. caldolyticus DNA fragments that specified proteolytic activity were obtained. Subcloning of one of these fragments in a protease-deficient *Bacillus subtilis* strain resulted in protease proficiency of the host. The nucleotide sequence of a 2-kb *Hinf*I-*Mlu*I fragment contained an open reading frame (ORF) that specified a protein of 544 amino acids. This ORF was denoted as the B. caldolyticus npr gene, because the nucleotide and amino acid sequences of the ORF were highly similar to that of the *Bacillus stearothermophilus* npr gene. Additionally, the size, pH optimum, and sensitivity to the specific Npr inhibitor phosphoramidon of the secreted enzyme indicated that the B. caldolyticus enzyme was a neutral protease. The B. stearothermophilus and B. caldolyticus enzymes differed at only three amino acid positions. Nevertheless, the thermostability and optimum temperature of the B. caldolyticus enzyme were 7 to 8-degrees-C higher than those of the B. stearothermophilus enzyme. In a three-dimensional model of the B. stearothermophilus Npr the three substitutions (Ala-4 to Thr, Thr-59 to Ala, and Thr-66 to Phe) were present at solvent-exposed positions. The role of these residues in thermostability was analyzed by using **site-directed mutagenesis**. It was shown that all three amino acid substitutions contributed to the observed difference in thermostability between the neutral proteases from B. stearothermophilus and B. caldolyticus.

L35 ANSWER 76 OF 87 MEDLINE on STN DUPLICATE 22 91260724 Document Number: 91260724. PubMed ID: 2046676. Beta-casein gene promoter activity is regulated by the hormone-mediated relief of transcriptional repression and a mammary-gland-specific nuclear factor.



Schmitt-Ney M; Doppler W; Ball R K; Groner B. (Friedrich Miescher Institute, Basel, Switzerland. ) MOLECULAR AND CELLULAR BIOLOGY, (1991 Jul) 11 (7) 3745-55. Journal code: 8109087. ISSN: 0270-7306. Pub. country: United States. Language: English.

- AB Transcription from the beta-casein **milk** protein gene promoter is induced by the synergistic action of glucocorticoid and prolactin hormones in the murine mammary epithelial cell line, HC11. We analyzed the binding of nuclear proteins to the promoter and determined their binding sites. **Site-directed mutagenesis** was used to determine the function of nuclear factor binding. During lactogenic hormone induction of HC11 cells, the binding of two nuclear factors increased. The binding of two other nuclear factors, present in uninduced cells, decreased. The basal activity of the promoter could be increased to and above the level of the induced wild-type promoter when the recognition sequences of the negatively regulated factors were mutated. This suggests that the beta-casein promoter is regulated by the relief of the repression of transcription. An essential tissue-specific factor was also found in nuclear extracts from the mammary glands of mice. Mutation of its recognition sequence in the beta-casein promoter led to the abolition of the induction of transcription by lactogenic hormones. The DNA sequences recognized by all five of these nuclear factors are conserved in the promoters of different casein genes from several species, confirming their importance in the regulation of **milk** protein gene transcription.

L35 ANSWER 77 OF 87 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN 91:366305 The Genuine Article (R) Number: FT582. BETA-CASEIN GENE PROMOTER ACTIVITY IS REGULATED BY THE HORMONE-MEDIATED RELIEF OF TRANSCRIPTIONAL REPRESSION AND A MAMMARY-GLAND-SPECIFIC NUCLEAR FACTOR. SCHMITTNEY M (Reprint); DOPPLER W; BALL R K; GRONER B. FRIEDRICH MIESCHER INST, POB 2543, CH-4002 BASEL, SWITZERLAND (Reprint). MOLECULAR AND CELLULAR BIOLOGY (1991) Vol. 11, No. 7, pp. 3745-3755. Pub. country: SWITZERLAND. Language: ENGLISH.

- \*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*
- AB Transcription from the beta-casein **milk** protein gene promoter is induced by the synergistic action of glucocorticoid and prolactin hormones in the murine mammary epithelial cell line, HC11. We analyzed the binding of nuclear proteins to the promoter and determined their binding sites. **Site-directed mutagenesis** was used to determine the function of nuclear factor binding. During lactogenic hormone induction of HC11 cells, the binding of two nuclear factors increased. The binding of two other nuclear factors, present in uninduced cells, decreased. The basal activity of the promoter could be increased to and above the level of the induced wild-type promoter when the recognition sequences of the negatively regulated factors were mutated. This suggests that the beta-casein promoter is regulated by the relief of the repression of transcription. An essential tissue-specific factor was also found in nuclear extracts from the mammary glands of mice. Mutation of its recognition sequence in the beta-casein promoter led to the abolition of the induction of transcription by lactogenic hormones. The DNA sequences recognized by all five of these nuclear factors are conserved in the promoters of different casein genes from several species, confirming their importance in the regulation of **milk** protein gene transcription.

L35 ANSWER 78 OF 87 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN 91277441 EMBASE Document No.: 1991277441. Modifying the substrate specificity of chymosin. Quinn D.; Pitts J.E.; Mantafounis D.; Lawler S.E.; Uusitalo J.; Penttila Strop M.P.. Lab. of Molecular Biology, Department of Crystallography, Birkbeck College, Malet Street, London WC1F 7HX, United Kingdom. Biochemical Society Transactions 19/3 (2678) 1991. ISSN: 0300-5127. CODEN: BCSTB5. Pub. Country: United Kingdom. Language: English.

L35 ANSWER 79 OF 87 CAPLUS COPYRIGHT 2003 ACS on STN

1992:35477 Document No. 116:35477 Protein engineering of the surface loops of chymosin. Orprayoon, Poonsook; Pitts, Jim E.; Nugent, Philip; Mantafounis, Dimitris; Lawler, Sean E.; Blundell, Tom L.; Usitalo, Janna; Penttila, Merja (Dep. Crystallogr., Birkbeck Coll., London, WC1E 7HX, UK). Biochemical Society Transactions, 19(3), 265S (English) 1991. CODEN: BCSTB5. ISSN: 0300-5127.

AB Suitable system for the development of protein engineering design principles in the area of food processing technol. The ability to engineer chymosin by recombinant DNA techniques, combined with structural and computer graphics anal., provide the key elements to the design cycle. Target surface loop was identified from residues 155-164 predicted to be suitable for replacement with both sequence from other family members and with novel loops of variable chain length. The insertion of this loop sequence from the enzyme Pencillopepsin into chymosin (PEN 154) and its expression in the fungus *Trichoderma reesei* is reported. Prochymosin A has previously been expressed and secreted using the fungus *T. reesei*. A signal peptide directs the correctly folded zymogen into the ferm. media under the control of the strong cbhl promoter of the cellobiohydrolase I gene. The low pH of the growth media leads to autocatalytic activation of the enzyme giving yields of 20-40 mgs/L. The mutation PEN 154 was introduced into the chymosin cDNA using an highly efficient **site -directed mutagenesis** method. The mutagenic 52-mer oligonucleotide was synthesized using phosphoramidite chem. and purified by reverse phase chromatog. on a FPLC system. The correct insertion was confirmed by DNA sequencing using the M13 dideoxy chain termination method of Sanger. The BamHI fragment contg. the PEN 154 mutation was cloned into the vector pAMH104-E deltaBam HI in the correct orientation. This was digested with EcoRI, dephosphorylated and combined with the large fragment of pAMH104 to produce the final chymosin B PEN 154 expression vector (pCHYB-PEN154). The *T. reesei* strain RUTC was transformed with pCHYB-PEN154 DNA, grow in liq. culture and assayed for **milk clotting activity**. The activities recorded were similar to those found for chymosin A and indicated that (i) the chymosin mutant was exported out into the media, (ii) the enzyme folded correctly with the PEN 154 loop, and (iii) it has high enzymic activity.

L35 ANSWER 80 OF 87 CAPLUS COPYRIGHT 2003 ACS on STN  
1991:96821 Document No. 114:96821 Recombinant manufacture of cysteine-substituted somatotropin analogs with increased biological activity. Parcells, Alan J.; Mott, John E.; Tomich, Che Shen C. (Upjohn Co., USA). PCT Int. Appl. WO 9008823 A1 19900809, 29 pp. DESIGNATED STATES: W: AU, DK, FI, HU, JP, KR, NO, SU, US; RW: AT, BE, CH, DE, ES, FR, GB, IT, LU, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1989-US5445 19891211. PRIORITY: US 1989-304733 19890131.

AB Analogs of somatotropin with one or more cysteine residues replaced by serine and having higher biol. specific activities than native somatotropin are prepd. by expression of the corresponding gene in *Escherichia coli*. A cDNA for bovine somatotropin was mutagenized by std. methods to replace 1, 2, or 4 cysteines with serines and the resulting genes expressed in *E. coli* using components of the trp regulon. The purified proteins were used to stimulate lactation in cattle and wt. gain in hypophysectomized rats. In cattle, injection of 5 or 15 mg somatotropin increased **milk yield** from 24.6 kg **milk** /day to 25 and 25.7 resp. Corresponding dosages of an analog with serine at positions 181 and 189 increased yields to 27.6 and 26.2 resp. The same analog increased wt. gain in hypophysectomized rats 1.78 fold over no hormone controls and 1.25-fold over bovine somatotropin controls.

L35 ANSWER 81 OF 87 CAPLUS COPYRIGHT 2003 ACS on STN  
1991:75822 Document No. 114:75822 Recombinant somatotropin analogs with a replaced amino acid residue on position 99 of the native mammalian somatotropin. Garlick, Robert L.; Lyle, Stephen B.; Mott, John E. (Upjohn Co., USA). PCT Int. Appl. WO 9008164 A1 19900726, 24 pp. DESIGNATED STATES: W: AU, DK, FI, HU, JP, KR, NO, SU, US; RW: AT, BE, CH, DE, ES, FR, GB, IT, LU, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO

- 1989-US5447 19891211. PRIORITY: US 1989-299107 19890119.
- AB Recombinant somatotropin analogs are prepd. by **site-directed mutagenesis** to replace the asparagine residue at position 99 of the native bovine somatotropin with .gtoreq.1 amino acid selected from proline, serine, glycine, Ser-Ser, or Ser-Asp. The recombinant somatotropins can be administered in an effective amt. to cows for enhancing the growth or increasing the **milk** prodn. The recombinant technique can also be used to make analogs of other somatotropins (e.g. porcine, fish, human, etc.). Thus, 3 recombinant somatotropin analogs were prepd. by replacing the asparagine residue at position 99 with glycine, serine, and aspartic acid, and administered i.m. to cows at 5 mg or 20 mg daily for 21 days. The 3.5% fat cor. **milk** yield (parameter for estg. **milk** prodn.) suggested that the **milk** yield of cows injected with the serine- or aspartic acid-substituted analog was greater than that of cows injected with glycine-substituted or native recombinant somatotropin. The relative potencies of wt. gain for rats injected with substituted analogs were 2.30-3.06-fold greater than the std. for the rats injected with native recombinant somatotropin. Furthermore, anal. by isoelec. focusing, SDS-PAGE, and reversed-phase HPLC before or after a 3-wk period of storage at -20.degree. showed the substituted analogs had a superior aq. stability relative to native recombinant somatotropin. The effective dosage range is 1.0-200 mg/day/animal; the most preferred dosage range is 5-50 mg/day/animal.
- L35 ANSWER 82 OF 87 CAPLUS COPYRIGHT 2003 ACS ON STN  
1990:404642 Document No. 113:4642 Stabilization of somatotropins by modification of cysteine residues utilizing **site directed mutagenesis** or chemical derivatization. Cady, Susan Mancini; Logan, John Steele; Buckwalter, Brian Lee; Stockton, Gerald W.; Chaleff, Deborah Tardy (American Cyanamid Co., USA). Eur. Pat. Appl. EP 355460 A2 19900228, 50 pp. DESIGNATED STATES: R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE. (English). CODEN: EPXXDW. APPLICATION: EP 1989-113852 19890727. PRIORITY: US 1988-236060 19880824; US 1989-372699 19890703.
- AB The present invention relates to novel modified or derivatized recombinant animal somatotropins. This invention also provides methods for stabilizing recombinant animal somatotropins by modification or deletion of the cysteine residues utilizing **site directed mutagenesis** to replace from one to four of the cysteine amino acid residues of said somatotropins with one or more different amino acid residues or by derivatization of (1) both cysteine amino acid residues in the small loop of said somatotropin, both cysteine amino acid residues in the large loop or the four cysteine amino acid residues in both loops of said somatotropin. Amino acid sequences of recombinant modified human, bovine, ovine, porcine, horse, and avian somatotropins are given. The new somatotropins are useful for improving the growth rate of meat-producing animals, the efficiency of feed utilization, increasing **milk** prodn. in lactating animals, and improving wool prodn. in sheep.
- L35 ANSWER 83 OF 87 MEDLINE ON STN DUPLICATE 23  
90338017 Document Number: 90338017. PubMed ID: 2116411. Effects of glycosylation on the secretion and enzyme activity of Mucor rennin, an aspartic proteinase of Mucor pusillus, produced by recombinant yeast. Aikawa J; Yamashita T; Nishiyama M; Horinouchi S; Beppu T. (Department of Agricultural Chemistry, Faculty of Agriculture, University of Tokyo, Japan.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1990 Aug 15) 265 (23) 13955-9. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.
- AB The Mucor rennin gene encoding a prepro form of the fungal aspartic proteinase from Mucor pusillus was expressed under the control of the yeast GAL7 promoter in Saccharomyces cerevisiae. The mature M. pusillus rennin secreted efficiently by yeast was a highly glycosylated protein. Analysis by a combination of **site-directed mutagenesis** of each of the three possible glycosylation sites and

treatment of the secreted *M. pusillus* rennins with endo-beta-N-acetylglucosaminidase H revealed that the mature yeast *M. pusillus* rennin contained two asparagine-linked glycosylation sites among the three possible glycosylation sites. A mutation of the 2 glycosylated asparagine residues of *M. pusillus* rennin resulted in significant decreases in the level of secretion by yeast cells. In addition, the extent of glycosylation of *M. pusillus* rennin was found to affect the enzyme properties such as milk-clotting and proteolytic activities.

- L35 ANSWER 84 OF 87 MEDLINE on STN DUPLICATE 24  
91149352 Document Number: 91149352. PubMed ID: 2290836. **Site-directed mutagenesis** reveals functional contribution of Thr218, Lys220 and Asp304 in chymosin. Suzuki J; Hamu A; Nishiyama M; Horinouchi S; Beppu T. (Department of Agricultural Chemistry, Faculty of Agriculture, University of Tokyo, Japan.) PROTEIN ENGINEERING, (1990 Oct) 4 (1) 69-71. Journal code: 8801484. ISSN: 0269-2139. Pub. country: ENGLAND: United Kingdom. Language: English.
- AB The functional contributions of amino acid residues Thr218 and Asp304 of chymosin, both of which are highly conserved in the aspartic proteinases, are analysed by means of **site-directed mutagenesis**. The optimum pH values, milk-clotting (C) and proteolytic (P) activities and kinetic parameters for synthetic oligopeptides as substrates were examined for the mutant enzymes. The mutation Thr18Ser caused a marked increase in the C/P ratio, which seemed to be due to a change in substrate recognition. Although the negative charge of Asp304 had been expected to play a role in lowering the optimum pH values in the aspartic proteinases, this turned out not to be the case in chymosin because both the mutations Asp304Ala and Asp304Glu caused a similar shift of the optimum pH towards the acidic side. In addition, the mutation Lys220Leu, which we generated previously, was found to cause a decrease in the C/P ratio, mainly due to the increase in the proteolytic activity.
- L35 ANSWER 85 OF 87 MEDLINE on STN DUPLICATE 25  
90094775 Document Number: 90094775. PubMed ID: 2689483. Cloning and sequencing of a complementary deoxyribonucleic acid coding for a bovine alpha s1-casein A from mammary tissue of a homozygous B variant cow. McKnight R A; Jimenez-Flores R; Kang Y; Creamer L K; Richardson T. (Department of Food Science and Technology, University of California, Davis 95616.) JOURNAL OF DAIRY SCIENCE, (1989 Oct) 72 (10) 2464-73. Journal code: 2985126R. ISSN: 0022-0302. Pub. country: United States. Language: English.
- AB A cDNA clone for bovine alpha s1-casein variant A was isolated from a mammary gland cDNA library using a synthetic degenerate oligonucleotide probe. The largest Pst I insert containing an EcoR I site was sequenced. It contained 1090 base pairs, 47 in the 5' noncoding region, 603 in the coding region and 440 in the 3' noncoding region. The nucleotide sequence was compared with three published cDNA sequences for alpha s1-casein variant B. The most obvious difference was the absence of the 39 bases encoding the 13 amino acids that are present in the B variant but absent from the A variant. In addition, five other single base positions differed within individual codons among the four sequences at the third base for each codon, but this did not change the amino acids encoded. There were, however, a number of differences found in the 3' noncoding region. The isolated cDNA was subjected to **site-directed mutagenesis** to replace a Val-Ile dipeptide with Phe-Phe to increase the chymosin sensitivity of the protein. When the milk proteins from mammary gland tissue extracts were typed, the alpha s1-casein A gene product was not detected.
- L35 ANSWER 86 OF 87 MEDLINE on STN DUPLICATE 26  
89315721 Document Number: 89315721. PubMed ID: 2501781. Alteration of catalytic properties of chymosin by **site-directed mutagenesis**. Suzuki J; Sasaki K; Sasao Y; Hamu A; Kawasaki H; Nishiyama M; Horinouchi S; Beppu T. (Department of Agricultural Chemistry,

Faculty of Agriculture, University of Tokyo, Japan. ) PROTEIN ENGINEERING,  
(1989 May) 2 (7) 563-9. Journal code: 8801484. ISSN: 0269-2139. Pub.  
country: ENGLAND: United Kingdom. Language: English.

- AB Artificial mutations of chymosin by recombinant DNA techniques were generated to analyze the structure--function relationship in this characteristic aspartic proteinase. In order to prepare the mutant enzymes in their active form, we established procedures for purification of correctly refolded prochymosin from inclusion bodies produced in *Escherichia coli* transformants and for its subsequent activation. **Mutagenesis** by linker insertion into cDNA produced several mutants with an altered ratio of **milk** clotting activity to proteolytic activity and a different extent of stability. In addition to these mutants, several mutants with a single amino acid exchange were also constructed by **site-directed mutagenesis** and kinetic parameters of these mutant enzymes were determined by using synthetic hexa- and octa-peptides as substrates. Exchange of Tyr75 on the flap of the enzyme to Phe caused a marked change of substrate specificity due to the change of *k<sub>cat</sub>* or *K<sub>m</sub>*, depending on the substrate used. Exchange of Val110 and Phe111 also caused a change of kinetic parameters, which indicates functional involvement of these hydrophobic residues in both the catalytic function and substrate binding. The mutant Lys220----Leu showed a marked shift of the optimum pH to the acidic side for hydrolysis of acid-denatured haemoglobin along with a distinct increase in *k<sub>cat</sub>* for the octa-peptide in a wide pH range.

L35 ANSWER 87 OF 87 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
1988:396352 Document No.: BA86:68991. PROTEIN ENGINEERING METHODOLOGY  
APPLICATIONS AND STATUS. JACKMAN R L; YADA R Y. DEP. FOOD SCI., UNIV.  
GUELPH, GUELPH, ON, CANADA N1G 2W1. FOOD BIOTECHNOL (N Y), (1987) 1 (2),  
167-224. CODEN: FBOIEE. ISSN: 0890-5436. Language: English.

- AB Developments in recombinant DNA (rDNA) technology have made selective alteration of primary amino acid sequences of proteins possible. Such manipulation using genetic engineering techniques has been referred to as protein engineering. Although this technology has seen only limited application in food research to date, this emerging and rapidly expanding technology offers exciting approaches to protein/enzyme modification studies, and may aid in the development of novel processes and/or more functional and nutritious proteinaceous foods. This review outlines some basic protein engineering methodology and strategies. In addition, examples are provided in which **site-directed mutagenesis** has been used advantageously in the specific modification of the enzymes subtilisin, lysozyme and chymosin, and of the casein proteins in **milk** and the storage proteins of potato tubers. These examples indicate the potential of protein engineering technology in investigations of structure-function relationships, stability, protein folding and conformation.

=> s egg  
L36 406787 EGG

=> s l36 and site directed mutagenesis  
L37 493 L36 AND SITE DIRECTED MUTAGENESIS

=> s l37 and IgE binding  
L38 2 L37 AND IGE BINDING

=> dup remove l38  
PROCESSING COMPLETED FOR L38  
L39 1 DUP REMOVE L38 (1 DUPLICATE REMOVED)

=> d l39 cbiab abs

L39 ANSWER 1 OF 1 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STNDUPLICATE 1  
2003098191 EMBASE Reduction of antigenicity and allergenicity of genetically

modified egg white allergen, ovomucoid third domain. Mine Y.;  
Sasaki E.; Zhang J.W.. Y. Mine, Department of Food Science, University of  
Guelph, Guelph, Ont. N1G2W1, Canada. ymine@uoguelph.ca. Biochemical and  
Biophysical Research Communications 302/1 (133-137) 28 Feb 2003.  
Refs: 26.

ISSN: 0006-291X. CODEN: BBRCA. Pub. Country: United States. Language:  
English. Summary Language: English.

AB Ovomucoid (Gal d1) is a major allergen in hen egg white,  
consisting of three tandem domains. In this study, five genetically  
modified third domain (DIII) mutants, which were substituted single or  
double amino acids within its IgE and IgG epitopes were compared with  
those prepared and their antigenicity and allergenicity with native  
analogue using Western immunoblot and enzyme-linked immunosorbent assay.  
The replacement of phenylalanine at 37 (F37) position with methionine  
caused drastical loss of IgG and IgE binding  
activities of human sera derived from egg allergic patients as  
well as disruption of the .alpha.-helix structure which comprises a part  
of the IgG and IgE epitopes. Substituting glycine at 32 position in  
conjunction with F37 showed a synergistic effect of decreasing  
antigenicity. The present study indicated that glycine 32 and  
phenylalanine 37 have an important role on its antigenicity and  
allergenicity as well as structural integrity of ovomucoid DIII. .COPYRG.  
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=> s grains  
L40 200190 GRAINS

=> s 140 and modified  
L41 3985 L40 AND MODIFIED

=> s 141 and IgE binding  
L42 0 L41 AND IGE BINDING

=> s 141 and anaphylaxis  
L43 1 L41 AND ANAPHYLAXIS

=> d 143 cblb abs

L43 ANSWER 1 OF 1 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN  
80082906 EMBASE Document No.: 1980082906. The demonstration of ingested wheat  
antigens in human breast milk. Kulangara A.C.. Immunol. Div., Post-grad.  
Inst. Bas. Med. Sci., Taramani, Madras, India. IRCS Medical Science 8/1  
(19) 1980.

AB CODEN: IRLCDZ. Pub. Country: United Kingdom. Language: English.  
It was decided to examine human breast milk for antigens of a common  
dietary component, wheat, using a modified Ouchterlony test  
employing sheep antisera which were non-reactive to human protein. Wheat  
grains were ground in a chilled mortar with 0.9% NaCl, allowed to  
settle. The supernatant (WG) was used as reference antigen, and gave 6  
lines (2 in large amount) against our antisera. 102 breast milk samples,  
obtained randomly in Madras maternity hospitals and post-natal clinics  
were tested, and of these 12 gave a major positive arc. Seventeen milk  
samples were further tested by the more sensitive technique of passive  
cutaneous anaphylaxis (PCA) in rat skin. A modified  
form of the test was used. Rats were given 0.5 ml i.v. of a 1:1 mixture of  
antiglutten serum and 2% Evans Blue in saline. Two hours later, the abdomen  
was depilated and intradermally injected with 0.05 ml amounts of saline,  
WG or milk. Saline blebs subsided rapidly and left normal skin. Wheals  
appeared in 2 min, and the rats were sacrificed at 30 min, skinned, and  
the inner surface of the skin photographed. WG and positive milks gave  
blue raised wheals 10-12 mm in diameter, showing induration. Sixteen of  
the 17 milk samples, randomly chosen, gave positive wheals. This  
preliminary result may indicate a much more general presence of quantities  
of wheat antigen too small to be demonstrable by Ouchterlony diffusion, or

of the presence of haptenic or monovalent antigenic fragments which would not give positive Ouchterlony tests but could be still reactive with the cell-fixed antibody in PCA. Six milk samples were titrated by 4-fold dilution tests in PCA and gave titers ranging from 1/4 to 1/32, showing blue wheals 12-8 mm in diameter.

=> s barley

L44 126403 BARLEY

=> s l44 and modified

L45 2079 L44 AND MODIFIED

=> s l45 and polynucleotide

L46 12 L45 AND POLYNUCLEOTIDE

=> dup remove l46

PROCESSING COMPLETED FOR L46

L47 9 DUP REMOVE L46 (3 DUPLICATES REMOVED)

=> d l47 1-9 cbib abs

L47 ANSWER 1 OF 9 CAPLUS COPYRIGHT 2003 ACS on STN

2002:676153 Document No. 137:212864 Identification, characterization, design and use of novel .alpha.-amylase. Callen, Walter; Richardson, Toby; Frey, Gerhard; Miller, Carl; Kazaoka, Martin; Mathur, Eric J.; Short, Jay M. (Diversa Corporation, USA). PCT Int. Appl. WO 2002068597 A2 20020906, 147 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US5538 20020221. PRIORITY: US 2001-PV270495 20010221; US 2001-PV270496 20010221; US 2001-PV291122 20010514.

AB The invention relates to .alpha.-amylases and to **polynucleotides** encoding the .alpha.-amylases. In addn. methods of designing new .alpha.-amylases and methods of use thereof are also provided. The .alpha.-amylases have increased activity and stability at increased pH and temp. The nucleotide sequence and the encoded amino acid sequence of an engineered .alpha.-amylase are provided. The enzyme can be used for starch liquefaction and other applications.

L47 ANSWER 2 OF 9 CAPLUS COPYRIGHT 2003 ACS on STN

2002:676145 Document No. 137:212862 Identification, screening, design, characterization and use of novel .alpha.-amylases. Callen, Walter; Richardson, Toby; Frey, Gerhard (Diversa Corporation, USA). PCT Int. Appl. WO 2002068589 A2 20020906, 301 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US5068 20020221. PRIORITY: US 2001-PV270495 20010221; US 2001-PV270496 20010221; US 2001-PV291122 20010514.

AB The invention relates to .alpha.-amylases and to **polynucleotides** encoding the alpha amylases. Identification and characterization of thermostable .alpha.-amylases and screening for .alpha.-amylase activity is described. The nucleotide and encoded amino acid sequences of the novel .alpha.-amylases are disclosed. In addn. methods of designing new

.alpha.-amylases and methods of use thereof are also provided. The .alpha.-amylases have increased activity and stability at acidic, and alk. pH and increased temp. Use of the .alpha.-amylase of the invention for starch liquefaction and other uses of the enzyme are disclosed.

L47 ANSWER 3 OF 9 CAPLUS COPYRIGHT 2003 ACS on STN  
2002:256481 Document No. 136:290008 Mutagenesis of plant 5-enol pyruvyl shikimate phosphate synthetase for stable enzyme expression in transgenic plant for glyphosate resistant. Warner, Simon Anthony James; Hawkes, Timothy Robert; Andrews, Christopher John (Syngenta Limited, UK). PCT Int. Appl. WO 2002026995 A1 20020404, 149 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-GB4131 20010914. PRIORITY: GB 2000-23911 20000929; GB 2000-23910 20000929; GB 2000-27693 20001113.

AB The present invention provides, inter alia, a glyphosate resistant EPSPS enzyme wherein in comparison with the wild type enzyme the EPSPS protein sequence is **modified** in that a first position is mutated so that the residue at this position is Ile rather than Thr and a second position is mutated so that the residue at this position is Ser rather than Pro, the mutations being introduced into EPSPS sequences which comprise the conserved region GNAGTAMRPL in the wild type enzyme such that **modified** sequence reads GNAGIAMRSL. The invention also includes glyphosate resistant plants regenerated from material transformed with **polynucleotides** which encode such EPSPS enzymes and a method of selectively controlling weeds in a field comprising such plants and glyphosate sensitive weeds, by the application to the field of glyphosate or an agronomically acceptable deriv.

L47 ANSWER 4 OF 9 CAPLUS COPYRIGHT 2003 ACS on STN  
2002:123055 Document No. 136:179298 **Polynucleotides** and polypeptides for hypersensitive response elicitor from Xanthomonas campestris, and their uses. Wei, Zhong-Min; Swanson, Shane S. (Eden Bioscience Corporation, USA). PCT Int. Appl. WO 2002012293 A2 20020214, 61 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US23787 20010727. PRIORITY: US 2000-PV224053 20000809; US 2001-829124 20010409.

AB The present invention is directed to an isolated DNA mol. encoding a Xanthomonas hypersensitive response elicitor protein or polypeptide. The DNA mol. and its encoded hypersensitive response elicitor protein or polypeptide have the following uses: imparting disease resistance to plants, enhancing plant growth, controlling insects on plants, imparting stress resistance, imparting post-harvest disease resistance, maximizing the benefit of or overcoming a yield penalty assocd. with a transgenic trait, inhibiting desiccation of cuttings from ornamental plants, promoting early flowering of an ornamental plant, and harvesting cuttings from ornamental plants. These can be achieved by applying the hypersensitive response elicitor in a non-infectious form to plants or plant seeds (or cuttings or fruits or vegetables harvested from such plants) or by expression of the hypersensitive response elicitor in transgenic plants. Expression vectors, host cells transgenic plants, transgenic plant cuttings, and transgenic plant seeds are also disclosed.



Genomic DNA encoding the hypersensitive response elicitor protein from *Xanthomonas campestris pelargonii* was cloned and the recombinantly expressed protein was active in tobacco. Results of Southern blot hybridizations with a gene hreX probe suggest that the gene is present in many *Xanthomonas* species.

- L47 ANSWER 5 OF 9 CAPLUS COPYRIGHT 2003 ACS on STN  
2002:51649 Document No. 136:67107 Growth stimulation vectors containing Leci1 and RepA genes improve transformation frequency in transgenic plants. Ross, Margit C.; Church, Laura A.; Hill, Patrea M.; Gordon-Kamm, William J.; Lowe, Keith S.; Hoerster, George J.; Bidney, Dennis L. (Pioneer Hi-Bred International, Inc., USA). PCT Int. Appl. WO 2002004649 A2 20020117, 33 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US21580 20010709. PRIORITY: US 2000-613094 20000710.
- AB A method of using genes that affect cell proliferation and cell death to increase the yield of transformed plant material, esp. from transformation refractory plants is described. The method comprises stably transforming a target cell with one or more vectors contg. at least one **polynucleotide** of interest. The genes may stimulate cell proliferation or passage through the cell cycle, or they may block apoptosis. Leci1 (leafy cotyledon) and wheat dwarf virus RepA genes were introduced into plasmids. The target cell has been previously **modified** to stimulate growth of the cell and has gone through at least one cell division.
- L47 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2003 ACS on STN  
2000:75681 Document No. 133:318309 Sequences of *Aureobasidium pullulans* glycogen branching enzymes and carbohydrates **modified** thereby. Taylor, Mark Andrew; Davies, Howard Vivian (Scottish Crop Research Institute, UK). PCT Int. Appl. WO 2000063399 A1 20001026, 49 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-GB1371 20000417. PRIORITY: GB 1999-8730 19990417; GB 1999-8731 19990417.
- AB There are provided novel cDNA **polynucleotide** sequences obtained from *Aureobasidium pullulans* which encode novel carbohydrate modifying enzymes. Host cells and in particular transgenic plants transfected with recombinant genetic constructs comprising the novel cDNA sequences are described. The enzymes encoded by the cDNA **polynucleotide** sequences may be used to produce carbohydrates or to modify existing carbohydrates, and this may be achieved in vitro or in vivo (for example by a transgenic plant). The **modified** carbohydrates so produced form a further aspect of the invention.
- L47 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2003 ACS on STN  
1998:795138 Document No. 130:62017 In situ modification of plant genes for improved herbicide resistance. Hawkes, Timothy Robert; Greenland, Andrew James; Evans, Ian Jeffrey (Zeneca Limited, UK). PCT Int. Appl. WO 9854330 A1 19981203, 49 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA,

GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English).  
CODEN: PIXXD2. APPLICATION: WO 1998-GB1499 19980522. PRIORITY: GB  
1997-11015 19970528.

AB A method of producing plants which exhibit an agronomically desirable trait comprises mutating or otherwise modifying in situ in a plant cell at least one gene which when **modified** is responsible for providing the said trait and regenerating from a cell exhibiting the said trait fertile morphol. normal whole plants. A **polynucleotide** is introduced into the plant cell, the said **polynucleotide** comprising at least one region which is substantially complementary to at least one region in the gene, which gene region when mutated or otherwise **modified** provides for the agronomically desirable trait. The region in the said **polynucleotide** contains at least one base mismatch in comparison with the like region in the said gene, so that the region in the said gene is altered by the DNA repair/replication system of the cell to include the said mismatch. The method is demonstrated by the use of mutagenic ribo/deoxyribo oligonucleotides specific for the 5-enolpyruvoylshikimate 3-phosphate (EPSP) synthase gene in *Brassica napus* for the provision of glyphosate resistance.

L47 ANSWER 8 OF 9 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STNDUPLICATE 1  
1998108186 EMBASE Electroporation and fusion **modified** by nucleic acids and complexes. Yu H.; Berg H.; H. Yu, Department of Physics, Nankai University, Tianjin 300071, China. Bioelectrochemistry and Bioenergetics 44/2 (227-232) 1998.

Refs: 14.

ISSN: 0302-4598. CODEN: BEBEEP.

Publisher Ident.: S 0302-4598(97)00070-6. Pub. Country: Switzerland.

Language: English. Summary Language: English.

AB The study of biopolymer **modified** electrofusion has been extended to nucleosides, nucleotides, **polynucleotides**, nucleic acids and their complexes with positively charged ligands (Ca<sup>2+</sup>, La<sup>3+</sup>, ethyldibromid, daunomycin, poly-L-lysine, histone). Whereas the mononucleotides show different effects on the electrofusion of **barley** protoplasts, the behaviour of the polymers are quite similar: at low concentrations the electrofusion was enhanced remarkably and at higher concentrations (> 0.05 mg ml<sup>-1</sup>) an inhibition effect was detected. Shielding the negative charge of phosphate groups the DNA complexes increase F(r) values to some extent. Quite strong enhancement of F(r) was also measured with nucleoproteins, e.g., nucleohistone. The essential process is the interaction between the polymers and the negatively charged protoplast membrane. This interaction was compared with the adsorption behavior of these polymers on the negative mercury electrode surface which can serve as a model for a 'half membrane'. With these results a first system of 'biopolymer **modified** electrofusion' is now available.

L47 ANSWER 9 OF 9 CAPLUS COPYRIGHT 2003 ACS on STN  
1948:38926 Document No. 42:38926 Original Reference No. 42:8264b-d Action of streptomycin on the germination of seeds of green plants and on **polynucleotides**. v. Euler, Hans; Bracco, Mario; Heller, Leo  
Compt. rend., 227, 16-18 (Unavailable) 1948.

AB Seeds of **barley**, *Secale cereale*, *Phleum pratense*, *Lolium perenne*, *Festuca elatior*, *F. duriuscula*, *Raphanus radicolica*, *Lactuca sativa*, and *Spinacia glabra* germinated on filter paper moistened with streptomycin (I) (greater than 2 mg./ml.) developed coleoptiles and first leaves that were completely white. With less concd. solns. of I, only the extremities of the leaves were green. Although I retards or arrests chlorophyll formation in the plastids of etiolated leaves, chlorophyll already formed in normal plants is not removed by I. The reaction of I with the nucleic acid and nucleoproteins of chondriosomes **modified** their differentiation and division and influenced the development of the chloroplasts.

=> s modified barley  
L48 0 MODIFIED BARLEY

=> s barley  
L49 126403 BARLEY

=> s L49 and modified  
L50 2079 L49 AND MODIFIED

=> s L50 and polynucleotide  
L51 12 L50 AND POLYNUCLEOTIDE

=> dup remove L51  
PROCESSING COMPLETED FOR L51  
L52 9 DUP REMOVE L51 (3 DUPLICATES REMOVED)

=> d L52 1-9 cbib abs

L52 ANSWER 1 OF 9 CAPLUS COPYRIGHT 2003 ACS on STN  
2002:676153 Document No. 137:212864 Identification, characterization, design  
and use of novel .alpha.-amylase. Callen, Walter; Richardson, Toby; Frey,  
Gerhard; Miller, Carl; Kazaoka, Martin; Mathur, Eric J.; Short, Jay M.  
(Diversa Corporation, USA). PCT Int. Appl. WO 2002068597 A2 20020906, 147  
pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR,  
BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB,  
GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK,  
LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL,  
PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US,  
UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE,  
BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT,  
LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN:

PIXXD2. APPLICATION: WO 2002-US5538 20020221. PRIORITY: US 2001-PV270495  
20010221; US 2001-PV270496 20010221; US 2001-PV291122 20010514.  
AB The invention relates to .alpha.-amylases and to **polynucleotides**  
encoding the .alpha.-amylases. In addn. methods of designing new  
.alpha.-amylases and methods of use thereof are also provided. The  
.alpha.-amylases have increased activity and stability at increased pH and  
temp. The nucleotide sequence and the encoded amino acid sequence of an  
engineered .alpha.-amylase are provided. The enzyme can be used for  
starch liquefaction and other applications.

L52 ANSWER 2 OF 9 CAPLUS COPYRIGHT 2003 ACS on STN  
2002:676145 Document No. 137:212862 Identification, screening, design,  
characterization and use of novel .alpha.-amylases. Callen, Walter;  
Richardson, Toby; Frey, Gerhard (Diversa Corporation, USA). PCT Int.  
Appl. WO 2002068589 A2 20020906, 301 pp. DESIGNATED STATES: W: AE, AG,  
AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ,  
DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN,  
IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK,  
MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL,  
TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY,  
KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE,  
DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN,  
TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US5068  
20020221. PRIORITY: US 2001-PV270495 20010221; US 2001-PV270496 20010221;  
US 2001-PV291122 20010514.

AB The invention relates to .alpha.-amylases and to **polynucleotides**  
encoding the alpha amylases. Identification and characterization of  
thermostable .alpha.-amylases and screening for .alpha.-amylase activity  
is described. The nucleotide and encoded amino acid sequences of the  
novel .alpha.-amylases are disclosed. In addn. methods of designing new  
.alpha.-amylases and methods of use thereof are also provided. The  
.alpha.-amylases have increased activity and stability at acidic, and alk.  
pH and increased temp. Use of the .alpha.-amylase of the invention for  
starch liquefaction and other uses of the enzyme are disclosed.

L52 ANSWER 3 OF 9 CAPLUS COPYRIGHT 2003 ACS on STN  
 2002:256481 Document No. 136:290008 Mutagenesis of plant 5-enol pyruvyl  
 shikimate phosphate synthetase for stable enzyme expression in transgenic  
 plant for glyphosate resistant. Warner, Simon Anthony James; Hawkes,  
 Timothy Robert; Andrews, Christopher John (Syngenta Limited, UK). PCT  
 Int. Appl. WO 2002026995 A1 20020404, 149 pp. DESIGNATED STATES: W: AE,  
 AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,  
 CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GH, GM, HR, HU, ID, IL, IN,  
 IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK,  
 MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ,  
 TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD,  
 RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI,  
 FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR.  
 (English). CODEN: PIXXD2. APPLICATION: WO 2001-GB4131 20010914.  
 PRIORITY: GB 2000-23911 20000929; GB 2000-23910 20000929; GB 2000-27693  
 20001113.

AB The present invention provides, inter alia, a glyphosate resistant EPSPS  
 enzyme wherein in comparison with the wild type enzyme the EPSPS protein  
 sequence is **modified** in that a first position is mutated so that  
 the residue at this position is Ile rather than Thr and a second position  
 is mutated so that the residue at this position is Ser rather than Pro,  
 the mutations being introduced into EPSPS sequences which comprise the  
 conserved region GNAGTAMRPL in the wild type enzyme such that  
**modified** sequence reads GNAGIAMRSL. The invention also includes  
 glyphosate resistant plants regenerated from material transformed with  
**polynucleotides** which encode such EPSPS enzymes and a method of  
 selectively controlling weeds in a field comprising such plants and  
 glyphosate sensitive weeds, by the application to the field of glyphosate  
 or an agronomically acceptable deriv.

L52 ANSWER 4 OF 9 CAPLUS COPYRIGHT 2003 ACS on STN  
 2002:123055 Document No. 136:179298 **Polynucleotides** and  
 polypeptides for hypersensitive response elicitor from Xanthomonas  
 campestris, and their uses. Wei, Zhong-Min; Swanson, Shane S. (Eden  
 Bioscience Corporation, USA). PCT Int. Appl. WO 2002012293 A2 20020214,  
 61 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR,  
 BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE,  
 GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS,  
 LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD,  
 SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM,  
 AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM,  
 CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT,  
 SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO  
 2001-US23787 20010727. PRIORITY: US 2000-PV224053 20000809; US  
 2001-829124 20010409.

AB The present invention is directed to an isolated DNA mol. encoding a  
 Xanthomonas hypersensitive response elicitor protein or polypeptide. The  
 DNA mol. and its encoded hypersensitive response elicitor protein or  
 polypeptide have the following uses: imparting disease resistance to  
 plants, enhancing plant growth, controlling insects on plants, imparting  
 stress resistance, imparting post-harvest disease resistance, maximizing  
 the benefit of or overcoming a yield penalty assocd. with a transgenic  
 trait, inhibiting desiccation of cuttings from ornamental plants,  
 promoting early flowering of an ornamental plant, and harvesting cuttings  
 from ornamental plants. These can be achieved by applying the  
 hypersensitive response elicitor in a non-infectious form to plants or  
 plant seeds (or cuttings or fruits or vegetables harvested from such  
 plants) or by expression of the hypersensitive response elicitor protein or  
 polypeptide in transgenic plants. Expression vectors, host cells transgenic plants,  
 transgenic plant cuttings, and transgenic plant seeds are also disclosed.  
 Genomic DNA encoding the hypersensitive response elicitor protein from  
 Xanthomonas campestris pelargonii was cloned and the recombinantly  
 expressed protein was active in tobacco. Results of Southern blot  
 hybridizations with a gene hreX probe suggest that the gene is present in

many *Xanthomonas* species.

- L52 ANSWER 5 OF 9 CAPLUS COPYRIGHT 2003 ACS on STN  
2002:51649 Document No. 136:67107 Growth stimulation vectors containing *Lec1* and *RepA* genes improve transformation frequency in transgenic plants. Ross, Margit C.; Church, Laura A.; Hill, Patrea M.; Gordon-Kamm, William J.; Lowe, Keith S.; Hoerster, George J.; Bidney, Dennis L. (Pioneer Hi-Bred International, Inc., USA). PCT Int. Appl. WO 2002004649 A2 20020117, 33 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MY, NZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US21580 20010709. PRIORITY: US 2000-613094 20000710.
- AB A method of using genes that affect cell proliferation and cell death to increase the yield of transformed plant material, esp. from transformation refractory plants is described. The method comprises stably transforming a target cell with one or more vectors contg. at least one **polynucleotide** of interest. The genes may stimulate cell proliferation or passage through the cell cycle, or they may block apoptosis. *Lec1* (leafy cotyledon) and wheat dwarf virus *RepA* genes were introduced into plasmids. The target cell has been previously **modified** to stimulate growth of the cell and has gone through at least one cell division.

- L52 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2003 ACS on STN  
2000:756881 Document No. 133:318309 Sequences of *Aureobasidium pullulans* glycogen branching enzymes and carbohydrates **modified** thereby. Taylor, Mark Andrew; Davies, Howard Vivian (Scottish Crop Research Institute, UK). PCT Int. Appl. WO 2000063399 A1 20001026, 49 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-GB1371 20000417. PRIORITY: GB 1999-8730 19990417; GB 1999-8731 19990417.
- AB There are provided novel cDNA **polynucleotide** sequences obtained from *Aureobasidium pullulans* which encode novel carbohydrate modifying enzymes. Host cells and in particular transgenic plants transfected with recombinant genetic constructs comprising the novel cDNA sequences are described. The enzymes encoded by the cDNA **polynucleotide** sequences may be used to produce carbohydrates or to modify existing carbohydrates, and this may be achieved in vitro or in vivo (for example by a transgenic plant). The **modified** carbohydrates so produced form a further aspect of the invention.

- L52 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2003 ACS on STN  
1998:795138 Document No. 130:62017 In situ modification of plant genes for improved herbicide resistance. Hawkes, Timothy Robert; Greenland, Andrew James; Evans, Ian Jeffrey (Zeneca Limited, UK). PCT Int. Appl. WO 9854330 A1 19981203, 49 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1998-GB1499 19980522. PRIORITY: GB 1997-11015 19970528.
- AB A method of producing plants which exhibit an agronomically desirable

trait comprises mutating or otherwise modifying in situ in a plant cell at least one gene which when **modified** is responsible for providing the said trait and regenerating from a cell exhibiting the said trait fertile morphol. normal whole plants. A **polynucleotide** is introduced into the plant cell, the said **polynucleotide** comprising at least one region which is substantially complementary to at least one region in the gene, which gene region when mutated or otherwise **modified** provides for the agronomically desirable trait. The region in the said **polynucleotide** contains at least one base mismatch in comparison with the like region in the said gene, so that the region in the said gene is altered by the DNA repair/replication system of the cell to include the said mismatch. The method is demonstrated by the use of mutagenic ribo/deoxyribo oligonucleotides specific for the 5-enolpyruvylshikimate 3-phosphate (EPSP) synthase gene in *Brassica napus* for the provision of glyphosate resistance.

L52 ANSWER 8 OF 9 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STNDUPLICATE 1  
1998108186 EMBASE Electroporation and fusion **modified** by nucleic acids and complexes. Yu H.; Berg H.. H. Yu, Department of Physics, Nankai University, Tianjin 300071, China. Bioelectrochemistry and Bioenergetics 44/2 (227-232) 1998.

Refs: 14.

ISSN: 0302-4598. CODEN: BEBEEP.

Publisher Ident.: S 0302-4598(97)00070-6. Pub. Country: Switzerland.

Language: English. Summary Language: English.

AB The study of biopolymer **modified** electrofusion has been extended to nucleosides, nucleotides, **polynucleotides**, nucleic acids and their complexes with positively charged ligands (Ca<sup>2+</sup>, La<sup>3+</sup>, ethyldiuronium, daunomycin, poly-L-lysine, histone). Whereas the mononucleotides show different effects on the electrofusion of **barley** protoplasts, the behaviour of the polymers are quite similar: at low concentrations the electrofusion was enhanced remarkably and at higher concentrations (> 0.05 mg ml<sup>-1</sup>) an inhibition effect was detected. Shielding the negative charge of phosphate groups the DNA complexes increase F(r) values to some extent. Quite strong enhancement of F(r) was also measured with nucleoproteins, e.g., nucleohistone. The essential process is the interaction between the polymers and the negatively charged protoplast membrane. This interaction was compared with the adsorption behavior of these polymers on the negative mercury electrode surface which can serve as a model for a 'half membrane'. With these results a first system of 'biopolymer **modified** electrofusion' is now available.

L52 ANSWER 9 OF 9 CAPLUS COPYRIGHT 2003 ACS on STN  
1948:38926 Document No. 42:38926 Original Reference No. 42:8264b-d Action of streptomycin on the germination of seeds of green plants and on **polynucleotides**. v. Euler, Hans; Bracco, Mario; Heller, Leo Compt. rend., 227, 16-18 (Unavailable) 1948.

AB Seeds of **barley**, *Secale cereale*, *Phleum pratense*, *Lolium perenne*, *Festuca elatior*, *F. duriuscula*, *Raphanus radiclecola*, *Lactuca sativa*, and *Spinacia glabra* germinated on filter paper moistened with streptomycin (I) (greater than 2 mg./ml.) developed coleoptiles and first leaves that were completely white. With less concd. solns. of I, only the extremities of the leaves were green. Although I retards or arrests chlorophyll formation in the plastids of etiolated leaves, chlorophyll already formed in normal plants is not removed by I. The reaction of I with the nucleic acid and nucleoproteins of chondriosomes **modified** their differentiation and division and influenced the development of the chloroplastids.

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